

Antibacterial and antioxidant properties of some selected egyptian plants

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Introduction.

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care that has been used by mankind all over the world in the form of folklore medicines [1]. According to the World Health Organization (WHO), approximately 25% of modern drugs used in the United States have been derived from plants [2]. In Egypt, many plants are used today in folk medicine and are sold at herbal vendors and shops [3]. The ancient Egyptians were familiar with many medicinal herbs and aware of their usefulness in the treatment of various diseases. They used the plant organs such as rhizomes, flowers, leaves, seeds, and oils. They applied their medicaments in the form of powders, pills, suppositories, creams, pastes, and ointments however, scientific evidence for the medicinal properties of such plants is not always demonstrated [4,5].

Phytochemicals (alkaloids, terpenoids, saponins, phenols and flavonoids) are biologically active chemical compounds in plants and act as a natural defense system for host plants and provide colour, aroma and flavor, these natural products or secondary metabolites have shown great potential in treating human diseases such as coronary heart diseases, diabetes, and infectious diseases [6].

Bacterial diseases are a type of infectious diseases caused by pathogenic bacteria and result when the harmful bacteria get into a body area, multiply, thrash the body's defensive mechanism, and emit toxins which damage cells and tissues that consequently results in the symptoms of bacterial disease [7]. Bacterial infection if untreated can lead to serious and life threatening complications such as sepsis, kidney and liver failure, toxic shock, and even death so it has always been considered as a major cause of morbidity and mortality in humans.

The antibiotics are substances that inhibit or suppress the growth and activity of microorganisms but the resistance to antibiotics is one of the biggest problems that faces public health [8,9]. There are various factors responsible to the emergence of resistance such as overuse of antibiotics, patient related factors, self-medications especially young adults etc. [10,11]. Due to rapid increase in the rate of infections, antibiotic resistance in microorganisms and side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs

because of their lesser side effects and low resistance in microorganisms [12,13].

The antioxidant defined as any substance that, when present in low concentration compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate" [14]. It is well known that oxidant by-products of normal metabolism such as free radicals and reactive oxygen species (ROS) in excess leading to various degenerative diseases of aging such as arthritis, asthma, dementia, cancer, cardiovascular disease, immune system decline, and brain dysfunction [15]. There are various endogenous and exogenous sources of antioxidants, the endogenous sources include antioxidant enzymes while exogenous sources include food sources and medicinal plants [16,17]. Studies on herbal plants have indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and anthocyanidins which may help to protect the cells against the oxidative damage caused by free-radicals [18,19]. Several synthetic antioxidants are used currently, but these substances may be inappropriate for chronic human consumption. Hence, the development of alternative antioxidants of natural origin has considerably increased. The medicines derived from plant products are safer than their synthetic counterparts [20,21].

The aim of this study is to investigate the antibacterial and anti-oxidant effect of the aqueous and organic extracts of some selected medicinal plants of Egypt.

Materials and methods

All chemicals used in this study were of analytical grade from Sigma Chemical Co. (St. Louis, MO, USA), BDH (Dorset, England) or Fluka Chemie Co. (Buchs, Switzerland).

Plant samples and extraction

Samples from a total 20 plant species belonged to 16 families (Table 1) were collected from a local market in Cairo, Egypt. These plants were selected on the base of their traditional use in Egypt folk medicine. The fresh samples were dried at 50°C, then reduced to fine particles using Waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before being used for analysis.

For aqueous extracts, 300 g dried ground plant material was soaked in 1L distilled water. The bottles containing soaked plant material were heated in water bath at 40°C for 2 hrs for hot water extract and for boiling water extract, the bottles were boiled for 30 min. The extracts were allowed to cool for about 6 hrs before it was rapidly filtered through Whatman No. 1 filter paper. For organic extract, 300 gm plant material was percolated with 1L ethanol and 1 L acetone in glass bottles, these bottles were vigorously shaken at a speed of 300 rpm, overnight. Plant residues were allowed to settle and the supernatant was filtered. All filtrates obtained were concentrated under reduced pressure (at 68°C) in a rotary evaporator to obtain the crude extract which kept at 4°C until further uses.

The percentage yield of extract for different solvents was calculated using the formula: Weight of final extract/ Weight of powdered X100.

Antibacterial activity

Microorganisms

The test organisms for *in-vitro* antibacterial screening were five reference microorganisms "*P. aeruginosa* (NCTC 10662), *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 12344), *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 10031) and five clinical isolated MDR bacteria "*S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *K. pneumonia* which isolated and identified by automated biochemical tests using Vitek®MS colorimetric identification card (bioMerieux - Marcy-l'Étoile, France). The susceptibility patterns were obtained using Vitek®MS aspartate aminotransferase. One single colony of each tested microorganism, taken from nutrient agar stock cultures into 10 mL sterile Muller-Hinton broth medium then incubated at 37°C for 16 - 20 hrs.

Disc diffusion assay [22]

10 µl of each plant extract (100 mg/mL), prepared by dissolving of 100 mg of crude extract in 1 mL DW in case of aqueous extracts and 1 mL DMSO in case of organic extracts, was applied to each filter paper disc (6 mm diameter) to give a final amount of 1 mg plant extract per disc. The discs were air dried and placed on top of the agar layer and incubated for 16-20 hrs at 37°C. Standard antibiotic discs served as positive control. All antimicrobial studies were done in triplicates.

Determination of MIC and MBC [23]

Serial dilution of each extract that showed significant zones of inhibition were individually placed in tubes labeled 1 to 10, each tube was then filled with 1 mL M-H broth including bacterial suspension. The resulting mixtures were incubated at 37°C for 24 hrs. Turbidity was taken as an indication of growth, and the lowest concentrations which did not show any turbidity was determined as MIC. In order to determine MBC values, 100 µL of the content of the tubes with no turbidity were cultured on the M-H agar medium and incubated at 37°C for 24 hrs.

Qualitative phytochemical analysis

Preliminary Phytochemical analysis for Flavonoids, Alkaloids, Glycosids, Terpenes, Phenolics, Saponins, Tannins were carried out using standard protocol as described by Ashfaq *et al.* [24].

DPPH assay [25]

The antioxidant activity of plant material was assayed by DPPH assay. 10 µl of plant extract (1mg/mL) was added to 100 µL of 0.2 mM DPPH solution in a microtitre plate. The reaction mixture was incubated at 25°C for 5 min, then measured at 520 nm. The DPPH without plant material served as the control. The methanol with respective plant extracts serves as blank. The percent DPPH scavenging activity was calculated as: $[(A_B - A_T) / A_B] \times 100$, where A_B and A_T are the absorbance of blank and plant material, respectively.

Statistical analysis

Six or more replicates were obtained for each treatment and the data are expressed as standard error of the mean. Comparisons between groups were performed by using paired students t-test on a Statistical Software Package

(SPSS). Differences were considered significant, if pvalue is less than 0.05 " $p < 0.05$ ".

Results and discussion

The average percentage of extraction yields with ethanol was better than acetone and distilled water as solvents. The maximum values were ethanol, acetone and aqueous extracts obtained from *Nigella sativa* (24.0, 23.7 and 21.4%, respectively), while the minimum values were aqueous and acetone extracts of *Zingiber officinale* (2.5 and 2.7%, respectively) (Fig 1).

The results of antimicrobial action showed that hot water extracts gave antibacterial effect better than boiling water extracts. By the same way, the ethanol extracts was better than acetone extracts. Against *P. aeruginosa* NCTC 10662 (Fig 2), the ethanol extract of *Citrullus colocynthis*, *Zingiber officinale*, *Cinnamomum zeylanicum*, and *Curcuma longa* produced greatest inhibition zones (16.83, 11.67, 11.17 and 11.0 mm, respectively) compared with standard antibiotics, only the aqueous and acetone extracts of *Cassia acutifolia* did not show antibacterial activity, while the growth of MβL, *P. aeruginosa* was inhibited by most plant extracts except 7 tested plants as shown in Fig 3. The maximum ones were acetone extract of *Zingiber officinale* and ethanol extract of both *Rosmarinus officinalis* and *Curcuma longa* (9.17, 9.0 and 8.8 mm, respectively). Cefotaxime and Cefaclor failed to show any effect at all although they were effective against *P. aeruginosa* NCTC 10662.

The ethanol extract of *Citrullus colocynthis* produced the greatest inhibition zone against *K. pneumonia* ATCC 10031 followed by acetone and ethanol extract of *Ficus carica* (17.0, 13.8 and 12.3 mm, respectively) (Fig 4), while some tested plants (*Cassia acutifolia*, *Foeniculum vulgare* and *Capsicum annum*) have no antibacterial effect at all. ESβL, *K. pneumonia* was resistant to many tested plants (Fig 5), but ethanol extract of *Citrullus colocynthis* and *Anastatica hierochuntica* produced the greatest effect (9.83 and 9.67 mm, respectively).

The ethanol extract of *Lepidium sativum* followed by *Citrus limon* produced the greatest inhibition zone (15.5 and 13.5 mm, respectively) against *E. coli* ATCC 25922 (Fig 6). Most tested plant extracts exhibited antibacterial effect against ESβL, *E. coli* except 4 tested plants as shown in Fig 7, the maximum inhibition zone produced by acetone extract of *Hibiscus sabdariffa* and ethanol extract of *Curcuma longa* (11.67 and 10.5 mm, respectively).

In Fig 8, both ethanol and hot water extract of *Punica granatum* and ethanol extract of *Citrus limon* produced the greatest inhibition zones against *S. aureus* ATCC 25923 (22.3, 16.8 and 15.5 mm, respectively). MRSA was sensitive to all tested plant extracts as shown in Fig 9 except *Capsicum annum* and *Cassia acutifolia*.

Acetone extract of *Citrullus colocynthis* followed by ethanol extract of *Citrus limon* produced the greatest inhibition zones against *S. pyogenes* ATCC 12344 (13.67 and 13.5 mm, respectively) (Fig 10). Against MDR, *S. pyogenes* in (Fig 11), some tested medicinal plants produced inhibition zones ranged from 7.17 to 9.17 mm. Acetone extract of *Punica granatum* and ethanol extract of

Mentha piperita were the best extracts as antibacterial agents (9.17 mm for both).

The MIC assay of *Curcuma longa* showed strong antagonistic activities against Gram-positive bacteria: MRSA (MIC 6.25 mg/mL), *S. aureus* ATCC 25923 (MIC 12.5 mg/mL) and *S. pyogenes* ATCC 12344, MDR *S. pyogenes* (each with MIC 25 mg/mL). For Gram-negative bacteria, the most common MIC value was 3.13 mg/mL for *E. coli* ATCC 25922, 6.25 mg/mL for *K. pneumonia* ATCC 10031, ES β L *E. coli* and *P. aeruginosa* NCTC 10662, and 12.5 mg/mL for M β L, *P. aeruginosa*. For *Lepidium sativum*, the MIC values ranged from 3.13 to 100 mg/mL and 1.56 to 200 mg/mL for *Cinnamomum zeylanicum*. *Hibiscus sabdariffa* showed MIC values ranged from 12.5 to 50 mg/mL, *Citrus limon* and *Foeniculum graecum*, each showed MIC values ranged from 12.5 to 100 mg/mL, and *Olea Europaea* showed MIC values ranged from 12.5 to 200 mg/mL (Table 2).

In case of *Curcuma longa*, slightly higher MBC values for ATCC *S. aureus* control strain (25 mg/mL; MIC 12.5 mg/mL) and MDR *S. pyogenes* (50 mg/mL; MIC 25 mg/mL) were obtained with its ethanol extract while by which, MBC and MIC values were identical for ATCC *E. coli*, ES β L *E. coli* (3.13 and 6.25 mg/mL, respectively) and NCTC *P. aeruginosa*, M β L, *P. aeruginosa* (6.25 and 12.5 mg/mL, respectively). MBC and MIC values of ethanol extract of *Lepidium sativum* were identical for ATCC *E. coli*, ATCC *S. aureus*, ES β L *E. coli*, and M β L, *P. aeruginosa* (3.13, 6.25, 12.5, and 25 mg/mL, respectively), while MBC values were slightly higher for both NCTC *P. aeruginosa* and MRSA (25 mg/mL; MIC 12.5 mg/mL) and ATCC *K. pneumonia* (12.5 mg/mL; MIC 6.25 mg/mL) (Table 2).

Phytochemical screening provide the presence of flavonoids, phenolics, saponins, tannins etc. at least in one extract of each tested plant as shown in Table 3.

All extracts produced high to moderate DPPH scavenging activity in most experimental plants. 5% of aqueous extracts and 10% of both ethanol and acetone extracts showed remarkable antioxidant activity ($\geq 90\%$ Inhibition), whereas 85% of ethanol extract and 90% of both aqueous and acetone extracts had moderate antioxidant activity ($< 90 - 40\%$ Inhibition). 5% of ethanol and aqueous extracts showed low antioxidant activity ($< 40\%$ Inhibition). The highest DPPH activities were observed in acetone extracts of *Citrus limon* and *Hibiscus sabdariffa* (94.8 and 93.9, respectively), and in ethanol extracts of *Citrus limon* and *Hibiscus sabdariffa*, (94.0 and 90.2, respectively), also observed in hot water extracts of *Citrus limon*, (91.8%) (Fig 12).

Medicinal plants have a long therapeutic history and still considered to be promising source of medicine in the traditional health care system. The plants possess chemotherapeutic, anti-microbial and anti-oxidant agents and act as a source of natural products useful in the development of novel drugs [26]. Extraction is the main step for recovering and isolating phyto-chemicals from tested plants and its efficiency is affected by the extraction method and the solvent used, moreover, the percentage yields of the extraction depend on the polarity of solvents,

pH, temperature and extraction time [27]. Water, ethanol and acetone are commonly used for the extraction and the properties of extracting solvents effect on the total phenolics content and anti-oxidant capacity [28,29]. Our results show that, the extraction yield increases with increasing polarity of the solvent used, this may be the reason why yields of ethanol, and acetone extracts are higher than yields of water. These results are in agreement with previous studies in which, extraction yields of rice bran and some medicinal plants decreased in the following order: ethanol > acetone > distilled water [30,31].

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new anti-bacterial agents for the treatment of bacterial infections is important. Our results indicated some plant extracts exhibited a good anti-bacterial activity and some others are limited as judged by their MIC values, these activities may be due to occurrence of different phyto-chemicals. The flavonoids and alkaloids have been found to possess anti-microbial and anti-oxidants properties in various studies [32,33]. Our extracts showed the larger inhibition zone as well as low MIC values against Gram-positive bacteria when compared with the Gram-negative bacteria. One of the most reasons is the different nature of cell wall among Gram-positive and Gram-negative bacteria however efflux pump system of Gram-negative bacteria may mediate for such difference [34,35]. It was noted that the hot water extract of the tested plants gave anti-bacterial effect better than boiling water which may be causes degradation of phyto-chemicals, also ethanol is a better solvents for more consistent extraction of anti-microbial substances from plants compared to water and acetone solvents [36]. Our results are supported by the study of Tomsone *et al.* [29], in which, he reported that the recovery of polyphenols from plant materials is influenced by the solubility of phenolic compounds in the solvent used. Ethanol stands as the first effective solvent, similar findings were reported previously [37].

In present study, ethanol extract of *Cassia acutifolia* was effective against most standered bacteria but did not show activity against MDR bacteria. Also Hamzah, [38] reported that ethanolic extract of *Cassia acutifolia* exhibited antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. This antibacterial activity is related to the presence of flavonoids [39]. Shahid *et al.* [26] reported that all extracts of *Nigella sativa* revealed significant antibacterial activity against all used bacteria, and the study carried out by Tanis *et al.* [40] stated that *E. coli*, *K. pneumonia* and *S. aureus* inhibited by *Nigella sativa*. Same result is reported by Mashhadian and Rakhshandeh [41] in which *Nigella sativa* inhibited *S. aureus* and *P. aeruginosa*. Ethanol extract of *Foeniculum vulgare* gave better inhibition zones as compared to its water and acetone extracts [26], also Kaur and Arora [42] reported that hot water and acetone seed extracts showed considerably good anti-bacterial activity against all bacteria except *K. pneumoniae* and *P. aeruginosa*, while present study revealed that ethanol extract showed antibacterial activity against *P. aeruginosa* NCTC 10662.

Niamsa and Sittiwet [43] reported that the aqueous extract of *Curcuma longa* demonstrated anti-bacterial activity against *S. epidermis*, *S. aureus*, *K. pneumoniae* and *E. coli*. Also like our results, Chakraborty *et al.* [44] reported that acetone extract of *Curcuma longa* was effective against *P. aeruginosa* and *E. coli*. *Zingiber officinale* extract demonstrated anti-bacterial activity against a variety of bacterial species including *H. pylori*, *S. aureus*, *P. aeruginosa* and *E. coli* [45]. In our results, all tested bacteria showed susceptibility toward ethanolic and acetone extracts of *Lepidium sativum*, while in previous study, *K. pneumoniae* was resistant [46]. Our results coincide with Sajjad *et al.* [47] that all extracts of *Punica granatum* exhibited a significant anti-bacterial activity against all tested organisms (*S. aureus*, *P. aeruginosa*, and *E. coli*). Dahham *et al.* [48] assessed the anti-microbial effect of ethanolic extract of seed, fruit, peel, and juice of pomegranate on specific bacteria and reported that the pomegranate peel extract had the greatest anti-microbial activity. Earlier studies have also shown the anti-bacterial activity of *Foenum graecum* extracts against *Klebsiella spp.*, *E. coli*, *P. aeruginosa*, and *S. aureus* [49,50]. In contrast to our result, anti-bacterial activity of water and alcoholic extracts of *Foenum graecum* seeds were very poor [51]. Previous study has provided that *Citrullus colocynthis* aerial part and fruit extracts exhibited good anti-microbial properties against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* [52,53].

We observed that the ethanolic extract of *Anastatica hierochuntica* showed anti-bacterial activities against tested bacteria. Such observation was supported by Al sobeai *et al.* [54] who found that ethanol extract of *Anastatica hierochuntica* showed anti-bacterial effect against both Gram positive (*S. aureus* and *S. pyogenes*) and Gram negative bacteria (*E. coli*, *P. aeruginosa* and *P. vulgaris*), while Mohamed *et al.* [55] reported that the extract of *Anastatica hierochuntica* was active only against *B. subtilis*. As our results, Keskin and Toroglu [56] reported that methanol extract of *Capsicum annuum* did not inhibit tested organisms except *P. aeruginosa*. Koffi-Nevry *et al.* [57] reported that methanol and aqueous extracts of *Capsicum annuum* was effective against only *S. aureus* but not effective against *P. aeruginosa* and *E. coli*, while Bokaeian *et al.*, [58] showed that the alcoholic extracts of *Capsicum annuum* had potent anti-microbial activity against ES β L, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

Keskin and Toroglu [56] observed the anti-bacterial activities of *Cinnamomum zeylanicum* extracts against *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *E. coli*. Moreover, Mandal *et al.* [59] reported the same observation against MRSA, *B. subtilis*, *S. aureus*, and *E. coli* which were the same tested bacteria that are inhibited by extraction of *Cinnamomum zeylanicum* in the study of Varalakshmi *et al.* [60]. The anti-microbial activity of methanol and water extracts of *Origanum marjorana* against *S. aureus*, *E. coli*, and *P. aeruginosa* was reported by Adam and Ahmed [61]. This result similar to Leeja and Thoppil [62] who reported that *Origanum marjorana* is bactericidal for each of *S. aureus*, *E. coli* and *P.*

aeruginosa. Also the study of Abdel-Massih *et al.* [63] showed that extract of *Origanum marjorana* and *Rosmarinus officinalis* exhibited more pronounced anti-bacterial activity on ES β L, *E. coli* than on *K. pneumoniae*.

According to the results obtained by Weckesser *et al.* and Genena *et al.*, [64,65], the rosemary extracts showed antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*. A similar results was reported by Pintore, *et al.* [66]. The present study showed anti-bacterial activity of different *Ficus carica* extracts against all tested bacteria, also ethanol extract of *Ficus carica* leaves exhibited anti-bacterial activity against *S. aureus*, *S. pyogenes* and *P. aeruginosa* while *K. pneumoniae* and *E. coli* appeared to be less sensitive [67]. These results are in agreement with other studies which showed beneficial properties and was even able to inhibit the growth of *S. aureus*, *S. epidermis*, *S. pyogenes*, *P. aeruginosa* and *E. coli* [68].

The present results are in fair correlation with the study carried out by Reuter *et al.* [69] in which garlic has been reported to inhibit the growth of *Staphylococcus* and many other species. In another study, garlic has been found to be high active against *E. coli* and *S. typhi* [70]. Sasaki *et al.* [71] found the garlic activity against MRSA, moreover, Karupiah and Rajaram [72] and Ross *et al.* [73] reported that all bacteria were susceptible to crude extracts of garlic except *Enterobacter sp.* and *Klebsiella sp.* The inhibitory activities exhibited by *Olea europaea* in this study support its use as anti-bacterial agent. This is in agreement with later studies by Ilias *et al.* [74] and Dada [75]. Also our results are in agreement with the study done by Pandey *et al.* [76] and Adedeji *et al.* [77] who reported the anti-microbial activity of *Citrus limon* extracts against *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *E. coli*, while in contrast to the present study, Hindi and Chabuck [78] reported that *Citrus limon* extract showed no effect against *E. coli*.

Sirag *et al.* [79] observed that *Hibiscus sabdariffa* extracts exhibited anti-bacterial activity against reference and MDR strains of *K. pneumoniae*, *S. aureus*, *E. coli* and *P. aeruginosa*. These microbes were also inhibited in our study as reported by other researchers [80,81]. Singh *et al.* [82] reported anti-bacterial activity of *Mentha piperita* extracts against *S. aureus*, *S. pyogenes*, *E. coli* and *K. pneumoniae*, the same results are reported by Priya *et al.* [83].

This discrepancy between the used plant extracts and the inhibiting degree of the tested strains can be explained by the fact that, the activity depends on the type, composition, and concentration of the plant extract, and the type of target micro-organisms [84]. Many other factors could also be involved such as age of the plant used, freshness of plant, physical factors as temperature and light, time of harvesting, drying method, the effect of solvents and the extraction method on the phenolic contents, and seasonal or intraspecific variation of plant extract composition [85,86]. Moreover, the negative results do not mean that the bioactive constituents are absent or that the plant is inactive, but active compounds may be present in insufficient quantities so that, the doses level would not be enough to exhibit the inhibitory effect. It is

also possible that the plant extracts may be active against other bacterial species that were not tested [46,87].

The most efficient anti-microbial plant extracts showed remarkable anti-oxidant activity, that may be refer to the polyphenolic components which could be responsible for both activities. The obtained results were in accordance with Ennajar *et al.* [88] and Lobna and Enas [89]. The previous study of kumar *et al.* [90] stated that presence of flavonoids, phenols, tannins, etc. in *Citrus limon*, and the work of Okereke *et al.* [91] indicated presence of tannins, saponins, flavonoids, etc in *Hibiscus sabdariffa*. Phytochemical screening assay of *Olea europea* indicated that presence of saponins, terpen, flavonoids and others [92]. Glycosides, flavonoids, saponins, etc were found in *Citrullus colocynthis* as reported by Ali *et al.* [93]. Baker *et al.* [94] reported the presence of tannins, flavanoids, glycosides, others in extracts of *Anastatica Hierochuntica*, and these results are in agreement with our results. Previous results showed higher phenolic compounds and others in ethanol extract of fenugreek seed [50]. Also flavonoids, phenols, saponins, terpenoids, and tannins, were found in different extracts of *Rosemarinus officinalis* [95]. The study by Khaleel *et al.* [96] reported that presence of flavonoid, alkaloid, glycosides, etc in *Punica granatum*. Our results compared favourable well with the one reported by Osabor *et al.* [97] who stated presence of alkaloids, saponins, flavonoids, etc in *Zingiber officinale* extracts, and with Ahmed *et al.* [98] who stated presence of alkaloids, carbohydrates, etc in *Lepidium sativum*. Previously, tannins, flavanoids, glycosides, phenols were reported in *Nigella sativa* extracts by Ishtiaq *et al.* [99]. Pandey *et al.* [100] reported that presence of alkaloid, saponin, flavanoide, and phenol in different extracts of the *Cinnamon* plant's bark, while protein and glycosides were absent, but in the present study, protein and glycosides were detected in all *Cinnamon* extracts. Our phytochemical screening assay of *Origanum majorana* revealed the presence of tannins, alkaloids, flavonoids, glycoside, saponin, carbohydrate, terpenes and phenolic compound [61] and similar results reported by Busatta *et al.* [101]. Rajesh *et al.* [102] reported the presence of alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids in alcoholic extract of *Curcuma longa*.

DPPH method is a preferred method in anti-oxidant assay because it is fast, easy and does not require a special reaction [103]. Our results are in agreement with Alothman *et al.* [104] and Zlotek *et al.* [105] who reported that the free radical scavenging potentials of both acetone and ethanol extracts are higher than hot water extracts. In a study conducted by Michiels *et al.* [28], acetone was more effective solvent than methanol for phenols extraction, however, the best solvent for phenolic extraction from horseradish roots was ethanol [29]. Moreover, the maximum polyphenols extraction was obtained in the alcoholic extract of *Bauhinia vahlii* followed by acetone, hot water and chloroform extracts [106].

References

[1] Kumari P., Joshi G C., Tiwari M L. Diversity and status of ethno-medicinal plants of Almora district in

Uttarakhand, India. Int J of Biodiversity and Conservation. 2011.3(7):298-326.

[2] Jinukuti MG., Giri A. Antimicrobial activity of phytopharmaceuticals for prevention and cure of diseases. Annals of Phytomedicine, 2013. 2(2):28-46.

[3] Abdel-Azim NS., Shams KA., Shahat AA., [et al.] Egyptian herbal drug industry: challenges and future prospects. Res J Med Plant. 2011. 5:136-144.

[4] Dagmar L. International trade in medicinal and aromatic plants, actors, volumes and commodities, plants. In: Bogers RJ, Craker LE, Lange D, eds. Medicinal and aromatic plants. 2006 Berlin, Heidelberg: Springer.

[5] Elezabeth DV., Subramanian A. Identification of phytochemical constituents and antimicrobial activity of Indigofera suffruticosa leaves. Int.J.Curr.Biotechnol, 2013. 1(7): 6-10.

[6] Chew YL., Chan EW., Tan PL. [et al.]. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complementary and Alternative Medicine, 2011. 11:1-10.

[7] Wrong Diagnosis website (2010) Accessed September 10, http://www.wrongdiagnosis.com/b/bacterial_diseases/intro.htm.

[8] Monte J., Abreu AC., Borges A., [et al.]. Antimicrobial Activity of Selected Phytochemicals against E. coli and S. aureus and Their Biofilms. Pathogens.2014. 3(2):473-498.

[9] Roca I, Akova M., Baquero F., [et al.]. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect, 2015. 6:22-29.

[10] Bhatia R., Narain JP. The growing challenges of antimicrobial resistance in the South-Asia Region. Indian J Med Res. 2010. 132(5):482-486.

[11] Khan F., Khan A., Kazmi SU. Prevalence and Susceptibility Pattern of Multi Drug Resistant Clinical Isolates of P. aeruginosa in Karachi. Pak J Med Sci. 2014. 30(5): 951-954.

[12] Seyyednejad SM., Motamedi HA. review on native medicinal plants in Khuzestan, Iran with antibacterial properties. Int J Pharmacol. 2010. 6(50): 551-560.

[13] Mani VM., Soundari AP., Karthiyaini D., [et al.]. Bioprospecting Endophytic Fungi and Their Metabolites from Medicinal Tree Aegle marmelos in Western Ghats, India. Mycobiology. 2015. 43(3): 303-310.

[14] Young IS., Woodside JV. Antioxidants in health and disease. J Clin Pathol.2001. 54:176-186.

[15] Li AN., Li S., Zhang UJ. [et al.]. Resources and Biological Activities of Natural Polyphenols. Nutrients. 2014. 6(12):6020-6047.

[16] Krishnaiah D., Sarbatly R., Nithyanandam RA. review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing. 2011. 89(3):217-233.

[17] Kotabagilu NP., Palvai VR., Urooj A. Protective Effect of Selected Medicinal Plants against Hydrogen Peroxide Induced Oxidative Damage on Biological Substrates. Int J Med Chem., 2014. ID.861084.V: 2014.

- [18] Narayanaswamy N., Balakrishnan KP. Evaluation of some Medicinal Plants for their Antioxidant Properties. *Int.J. PharmTech Res.* (2011). 3(1) 381-385.
- [19] Moustafa SM., Menshawi BM., Wassel GM. [et al.]. Screening of some Wild and Cultivated Egyptian Plants for their Free Radical Scavenging Activity. *Int.J. PharmTech Res.* 2014. 6(4):1271-1278.
- [20] Nan WU., Kuang FU. Yu-Jie Z., [et al.]. Antioxidant Activities of Extracts and Main Components of *Cajanus cajan* (L.) Millsp. Leaves. *Molecules.* (2009) 14:1032-1043.
- [21] Wannas WA., Mhamdi B., Sriti J., [et al.]. Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. *italica*) leaf, stem and flower. *Food Chem Toxicol.* 2010. 48:1362-1370.
- [22] Senthil-Rajan D., Rajkumar M., Srinivasan R., [et al.]. Investigation on antimicrobial activity of root extracts of *Thespesia populnea* Linn. *Tropical Biomedicine.* 2013. 30(4): 570–578.
- [23] Mahboubeh I., Marziyeh S., Françoise B., [et al.]. Antimicrobial Activity of *Glycyrrhiza glabra* L. Iranian J of Phar Res. 2010. 9 (4): 425-428.
- [24] Ashfaq M., Shah KW., Ahmad S. [et al.]. Preliminary phytochemical screening of alcoholic & aqueous extracts of *Mentha longifolia* linn. Leaves. *Int J of Biolog & Pharm Res.* 2012. 3(3): 384-386.
- [25] Mensor LL., Menezes FS., Leitão GG., [et al.]. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phyther. Res.* 2001. 15: 127-130.
- [26] Shahid W., Durrani R., Iram S., [et al.]. Antibacterial activity in Vitro of medicinal plants. *Sky J of Microbiology Research.* 2013. 1(2); 5–21.
- [27] Diem Do Q., Angkawijaya AE. Tran-Nguyen, P. L. Huynh, L. H. Soetaredjo, F. E. Ismadji, S. Ju Y. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic.* J of food and drug analysis. 2014. 2: 296-302.
- [28] Michiels, J. A., Kevers, C., Pincemail, J. [et al.]. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chemistry.* 2012. 130(4); 986-993.
- [29] Tomsone L., Kruma Z., Galoburda R. Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (*A Armoracia rusticana*). *World Acad. Sci. Eng. Technol.* 2012. 6(4), 903–908.
- [30] Sultana B., Anwar F., Ashraf M. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules.* 2009. 14:2167e80.
- [31] Sulaiman, C. T., Shahida, V., Balachandran I. Effect of Extraction Solvent on the Phytoconstituents of *Aegle marmelos* (L.) Correa. *J of Natural Remedies.* 2015. 15(1):58-64.
- [32] Amara AA., El-Masry MH., Bogdady HH. Plant crude extracts could be the solution: extracts showing in vivo antitumorigenic activity. *Pakistan Journal of Pharmaceutical Sciences.* 2009. 21(2):159-171.
- [33] Javid T., Adnan M., Tariq A., [et al.]. Antimicrobial activity of three medicinal plants (*Artemisia indica*, *Medicago falcate* and *Tecoma stans*. *Afr J Tradit Complement Altern Med.*, 2015.12(3):91-96.
- [34] Li XZ., Nikaido H. Efflux-mediated drug resistance in bacteria: an update, *Drugs.* 2009 69(12);1555–1623.
- [35] Marasini BP., Baral P., Aryal P., [et al.]. Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria. *BioMed Research International*, 2015 ID 265425, 6 pages, <http://dx.doi.org/10.1155/2015/265425>.
- [36] Azwanida NA. Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants.* 2015. 4(3):196.
- [37] Nićiforović, N., Mihailović, V., Mašković, P., [et al.]. Antioxidant activity of selected plant species; potential new sources of natural antioxidants”, *Food and Chemical Toxicology.* 2010. 48(11); 3125-3130.
- [38] Hamzah AM. Antibacterial of *Cassia acutifolia* extract against some bacteria and anti-biofilm formation activities against *Pseudomonas aeruginosa*. *Int. J. of Humanities, Arts, Med. and Sci.* 2016. 4(9); 2454-4728.
- [39] Kurkin VA., Shmygareva AA. The development of new approaches to standardization of *Cassia acutifolia* leaves. *J of Pharmacognosy and Phytochemistry.* 2014. 3 (3): 163-167.
- [40] Tanis H., Aygan A., Digrak M. Antimicrobial Activity of Four *Nigella* Species Grown in Southern Turkey. *Int. J. Of Agri. & Biol.*, 2009. 11(6):771-774.
- [41] Mashhadian NV., Rakhshandeh H. Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aeruginosa* and *C. albicans*. *Pak. J. Med. Sci.*, 2005. 21(1):47–52.
- [42] Kaur GJ., Arora DS. Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae - Current status. *J. of Medi. Plants Res.*, 2010. 4(2):87-94.
- [43] Niamsa N., Sittiwet C. Antimicrobial activity of *Curcuma longa* aqueous extract. *J. of Pharmacology and Toxicology.* 2009. 4(4); 173–177.
- [44] Chakraborty B., Nath A., Saikia H., [et al.]. Bactericidal activity of selected medicinal plants against multidrug resistant bacterial strains from clinical isolates. *Asian Pac J Trop Med.* 2014. 7(1): 435-441.
- [45] Chrubasik S., Pittler M., Rougogalis B., Zingiberis rhizome: a comprehensive review of ginger effect and efficiency profiles. *Phytomed.* 2005. 2: 634-701.
- [46] Akrayi HS., Tawfeeq JD. Antibacterial activity of *Lepidium sativum* and *Allium porrum* extracts and juices against some gram positive and gram negative bacteria. *Medical Journal of Islamic World Academy of Sciences.* 2012. 20(1):10-16.
- [47] Sajjad W., Sohail M., Ali B., [et al.] Antibacterial activity of *Punica granatum* peel extract. *Mycopath.* 2015. 13(2): 105-111.
- [48] Dahham SS., Ali MN., Tabassum H. [et al.]. Studies on antibacterial and antifungal activity of (*Punica granatum* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, 2010. 9(3);273–281.

- [49] Premanath R., Sudisha J., Lakshmi Devi N., [et al.]. Antibacterial and Anti-oxidant Activities of Fenugreek (*Trigonella foenum graecum* L.) Leaves. Research Journal of Medicinal Plants, 2011. 5: 695-705.
- [50] Mishra R., Mandloi S., Yadav N., [et al.]. Phytochemical analysis of *Trigonella foenum graecum* and its antibacterial activity against *Staphylococcus aureus*. World J. of pharmacy and pharmaceutical sci. 2016. 5(6);1408-1423.
- [51] Kroum NH. Antibacterial Activity of *Trigonella foenum graecum* linn (Fenugreek) Seed -Extracts Against Some Species of Enterobacter. M.Sc. Thesis, faculty of Public Health and Environmental Hygiene. 2009. University of Khartoum.Sudan.
- [52] Bnyan, I., Hasan, H., Ewadh M. Antibacterial Activity of *Citrullus Colocynthis* against different types of bacteria. Advances in Life Science and Technology. 2013. www.iiste.org ISSN 2224-7181, Vol 7.
- [53] Shawkey AM., Rabeh MA., Abdellatif AO. Biofunctional molecules from *Citrullus colocynthis*: An HPLC/MS analysis in correlation to antimicrobial and anticancer activities. Advances in Life Science and Technology. 2014). www.iiste.org ISSN 2224-7181 Vol. 17.
- [54] Al Sobeai, S. M., Al-Ghanayem, A. A., Alhussaini, M. S., [et al.]. Antibacterial Activity of *Anastatica hierochuntica* L. Extracts Against Different Groups of Bacterial Pathogens: An in- vitro test. Aust. J. Basic & Appl. Sci. 2015. 9(36):27-30.
- [55] Mohamed AA., Khalil AA., El- Beltagi HS. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaena thebaica*). Grasas Y Aceites, 2010. 61(1): 67-75.
- [56] Keskin D., Toroglu S. Studies on antimicrobial activities of solvent extracts of different spices. J Environ Biol.; 2011. 32(2): 251-256.
- [57] Koffi-Nevry R., Kouassi KC., Nanga ZY. [et al.]. Antibacterial Activity of Two Bell Pepper Extracts: *Capsicum annum* L. and *Capsicum frutescens*. Journal International Journal of Food Properties. 2012.15(5); 961-971.
- [58] Bokaeian M., Saeidi S., Bazi S., [et al.]. The Effects of *Capsicum annum* L. Extract on the Control of Single and Dual Biofilms of Common Pathogenic Strains Causing Urinary Tract Infection. ZJRMS, 2014. 16(10): 65-68.
- [59] Mandal S., DebMandal M., Saha K., [et al.]. In vitro Antibacterial Activity of three Indian Spices against Methicillin-Resistant *Staphylococcus aureus*. Oman Med. J. 2011. 26:319–323.
- [60] Varalakshmi B., Vijayaanand A. Karpagam T., [et al.]. In vitro antimicrobial and anticancer activity of *Cinnamomum zeylanicum* linn bark extracts. Int J Pharm Pharm Sci, 2013. 6(1); 12-18.
- [61] Adam SIY., Ahmed TG. Phytochemical Screening and Biological effect of Indigenous Medicinal plant *Origanum majorana* extracts. Journal of Faculty of Science and Technology, 2014. (5).
- [62] Leeja L., Thoppil JE. Antimicrobial activity of methanol extract of *Origanum majorana* L.(Sweet marjoram). J of Environmental Biol, 2007. 28(1): 145-146.
- [63] Abdel-Massih R., Abdou E., Baydoun E., [et al.]. Antibacterial Activity of the Extracts Obtained from *Rosmarinus officinalis*, *Origanum majorana* and *Trigonella foenum-graecum* on Highly Drug-Resistant Gram Negative Bacilli. J of Botany, 2010 Article ID 464087, 8 pages.
- [64] Weckesser S., Engel K., Simon-Haarhaus B., [et al.]. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. Phytomedicine, 2007. 14(7-8):508-516.
- [65] Genena AK., Hense H., Junior AS., [et al.]. Rosemary (*Rosmarinus officinalis*) – a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Ciênc. Tecnol. Aliment., Campinas, 2008. 28(2): 463-469.
- [66] Pintore G., Usai M., Brade P., [et al.]. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour and Fragrance J., Sassari/Ajaccio, 2002. 17(1):15-19.
- [67] Rashid KI., Mahdi NM., Alwan MA., [et al.]. Antimicrobial activity of fig (*Ficus carica* Linn.) leaf extract as compared with latex extract against selected bacteria and fungi. J of Babylon University/Pure and Applied Sci, 2014. 22(5).
- [68] Jeong MR., Kim HY., Cha JD. Antimicrobial activity of methanol extract from *Ficus carica* leaves against bacterial. J. Bacteriol. Virol. 2009. 39: 97-102.
- [69] Reuter HD., Koch HP., Lawson DL. Therapeutic effects and applications of Garlic and its preparations in: Garlic: The Science and therapeutic actions of *Allium sativum* L. and related species, 2nd Edn , Koch , H.P. and D.L. Lawson (Eds): Wilkins, Baltamare , MD.; 1996. PP135-213.
- [70] Abdou A., Abou-Zaid AA., Al-Sherneeny MR., [et al.]. Anti-microbial activities of *Allium sayivum*, *Allium cepa*, *Rephanus sayivus*, *Capsicum frutescens*, *Eruca sativa* and *Allium kurret* on bacteria “ Plant foods for Human Nutrition, 1972. 22(29-35):485-499.
- [71] Sasaki JT., Kita K., Ishita H., Uchisasawa, H., Matusé, H. Anti-bacterial activity of garlic powder against *Escherichia coli* 0-157, J. Nutr. Sci.Vitaminol. 1999. 45:785-790.
- [72] Karuppiyah P., Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific Journal of Tropical Biomedicine. 2012. 2(8):597-601.
- [73] Ross ZM., O'gara EA., Hill DJ., [et al.]. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microb.2001. 67:475–480.
- [74] Ilias F., Kholkhal W., Gaouar N., [et al.]. Antibacterial and antifungal Activities of Olive (*Olea europaea* L.) from Algeria. J. Microbiol. Biotech. Res., (2011) 1(2); 69-73.
- [75] Dada EO. Antibacterial Activities of *Olea europaea* Leaf Extract on Some Bacteria Isolated from a Refused Dump Site in Akure, Nigeria. Journal of Biology, 2013. 1(6):118-124.

- [76] Pandey A., Kaushik A. [et al.]. Evaluation of antimicrobial activity and phytochemical analysis of Citrus limon. JPBMS, 2011. 13(17).
- [77] Adedeji GB., Fagade E., Oyelade AA. Prevalence of *Pseudomonas aeruginosa* in clinical samples and its sensitivity to citrus extrats. African J Bio Res, 2007. 10.183-187.
- [78] Hindi KN., Chabuck GZ. Antimicrobial activity of different aqueous lemon extracts. J Appl Pharm Sci, 2013. 3 (6).074-078.
- [79] Sirag N., Ahmed EM., Algaili AM., [et al.]. Antibacterial Activity of Roselle (*Hibiscus Sabdariffa* L) Calyx Extract. Int J of Indigenous Medi Plants, 2013. 46(4);1487-1491.
- [80] Mei-Chi Y., Che-Yi C. Anti-campylobacter, anti-aerobic and antioxidative effects of roselle calyx extract and protocatechuic acid in ground beef. Int J of Food Micro, 2008. 127: 73-77.
- [81] Alaga TO., Edema MO., Atayese AO., [et al.]. Phytochemical and in vitro anti-bacterial properties of *Hibiscus sabdariffa* L juice. J. Med. Plants Res. 2014. 8(6);339-344.
- [82] Singh R., Shushni MA., Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. Arabian J of Chemistry, 2015. 8(3);322-328.
- [83] Priya AM., Manikandan M., Kalaiselvi G., [et al.]. Screening of antibacterial activity of *Mentha piperita* L. Asian J. Microbiol. Biotechnol. Environ. Sci. 2007. 9(4):1049-1052.
- [84] Marino M., Bersani C., Comi G. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae, Int J of food Micro. 2001. 67,187-195.
- [85] Hayouni EA., Chraief I., Abedrabba M., [et al.]. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. International Journal of Food Microbiology. 2008.125(3), 242-251.
- [86] Jouda MM., Elbashiti T., Masad A., [et al.]. Synergistic effect of *Ficus sycomorus* (Moraceae) leaf and stem-bark extracts against Some Selected Pathogens. Int J of Sci and Research Publications, 2015. 5(12):492-496.
- [87] Parekh J., Chanda SV. Antibacterial Activity of Aqueous and Alcoholic Extracts of 34 Indian Medicinal Plants against Some *Staphylococcus* Species. Turkish Journal of Biology. 2008. 32:63-71.
- [88] Ennajar M., Bouajila J., Lebrihi A., [et al.]. The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils, J. Sci. Food Agric., 2010. 3: 462-470.
- [89] Lobna SN., Enas AA. Screening of antibacterial and antifungal activities of five medicinal plants from Saudi Arabia environment. J. Appl. Sci. Res., 2012. 8(4): 2356-2365.
- [90] kumar KA., Narayani M., Subanthini A., [et al.]. Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels -Utilization of Fruit Waste. International Journal of Engineering Science and Technology, 2011. 3(6):5414- 5421.
- [91] Okereke CN., Iroka FC., Chukwuma MO. Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa*. Int. J. of Herbal Medicine; 2015. 2(6):16-19.
- [92] Nora NB., Hamid K., Snouci M. Antibacterial Activity and Phytochemical Screening of *Olea Europaea* Leaves from Algeria .The Open Conference Proceedings Journal, 2012. 3:66-69
- [93] Ali AA., Alian MA., Elmahi HA. Phytochemical Analysis of Some Chemical Metabolites of *Colocynth* Plant (*Citrullus colocynthis*) and its Activities as Antimicrobial and Antiplasmodial. J. Basic. Appl. Sci. Res.,2013. 3(5):228-236.
- [94] Baker RK., Mohammd TU., Ali BH., Jameel, N. M. The Effect Of Aqueous Extract Of *Anastatica Hierochuntica* On Some Hormones In Mouse Females. Ibn Al-Haitham Jour. for Pure & Appl. Sci. 2013. 26(2);198-205.
- [95] Johar S., Irfan S., Ahmed S., Jabeen, R. Phytochemical screening and antibacterial activity of *Rosmarinus officinalis* against *Escherichia coli*. local isolates. Int. J. of Basic and Applied Sci., 2015. 4(4): 413-421.
- [96] Khaleel AI., Sijam,K., Rashid TS. [et al.]. Phytochemical determination and antibacterial activity of *Punica granatum* peel extracts against Plant pathogenic bacteria. American J. of Plant Sci., 2016. 7; 159-166.
- [97] Osabor VN., Bassey FI., Umoh UU. Phytochemical Screening and Quantitative Evaluation of Nutritional Values of *Zingiber officinale*. American Chem. Sci. J.,2015. 8(4): 1-6.
- [98] Ahmad R., Mujeeb M., Anwar F., [et al.]. Pharmacognostical and phytochemical analysis of *Lepidium sativum*. seeds. Int. Current Pharma J, 2015. 4(10):442-446.
- [99] Ishtiaq S., Ashraf M., Hayat MQ., [et al.]. Phytochemical Analysis of *Nigella sativa* and its Antibacterial Activity against Clinical Isolates Identified by Ribotyping. Int. J. Agric. Biol., 2013. 15(6).
- [100] Pandey S., Pandey R., Singh R. Phytochemical Screening of Selected Medicinal Plant Cinnamon *Zeylanicum* bark extract, Area of research; Uttarakhand, India. Int. J. of Sci. & Res. Pub., 2014. 4 (6):1-6.
- [101] Busatta C., Vidal RS., Popiolski AS. [et al.] Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. Food Microbiology. 2008. 25: 207-211.
- [102] Rajesh H., Rao SN., Megha RN., [et al.]. Phytochemical analysis of methanolic extract of *Curcuma longa* linn rhizome. Int. J. of Universal Phar. and Bio Sci. 2013. 2(2): 39-45.
- [103] Aksoy L., Kolay E., Agilonu Y., [et al.]. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. Saudi J of Biol Sci. 2013. 20:235-239.
- [104] Alothman M., Bhat R., Karim A. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 2009. 115:785-788.

[105] Złotek U., Mikulska S. Nagajek M. [et al.]. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L) extracts. Saudi Journal of Biological Sciences. 2014. 23: 628–633.

[106] Sowndhararajan K., Kang SC. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. Saudi J. Biol. Sci. 2013. 20:319–325.

ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF SOME SELECTED EGYPTIAN PLANTS

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Introduction. Medicinal plants have been used as a source of therapies since ancient times in Egypt. The present study was designed to investigate the anti-bacterial and anti-oxidant activity of different extracts from 20 selected medicinal plants of Egypt.

Materials and methods. The disk diffusion method followed by microbroth dilution were used to determine minimum inhibitory concentration of the plant extracts against 10 bacterial strains belonging to 5 species, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*. While qualitative phytochemical screening followed by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay were used to assess the anti-oxidant of the extracts.

Results. The results indicated that all studied crude extracts were able to inhibit the growth of at least three of the tested bacteria. Moreover, all studied plants have various bioactive phyto-chemicals and were observed to be high to moderate antioxidant agents.

Conclusion. Finally, the target of this paper is to describe the most interesting plant extracts investigated here to be alternative medicines.

Key words: Anti-bacterial; Anti-oxidant; Medicinal plants; Phyto-chemicals; DPPH.

Table 1. Selected medicinal plants for screening their biological activities.

Family	Scientific name (Code)	Common name	Plant parts
Leguminosae	<i>Foeniculum graecum</i>	Fenugreek	Seeds
	<i>Cassia acutifolia</i>	Alexandrian Senna	Dried leaflets, pods
Ranunculaceae	<i>Nigella sativa</i>	Black seeds	Seeds
Umbelliferae	<i>Foeniculum vulgare</i>	Fennel	Seeds
Brassicaceae	<i>Anastatica hierochuntica</i>	Mary's hand	Branches, seeds, bods
	<i>Lepidium sativum</i>	Garden cress	Seeds
Lythraceae	<i>Punica granatum</i>	Pomegranate	Rind of the fruits
Zingiberaceae	<i>Curcuma longa</i>	Turmeric	Dried rhizome
	<i>Zingiber officinale</i>	Ginger	Roots
Solanaceae	<i>Capsicum annum</i>	Red pepper	Fruits
Lauraceae	<i>Cinnamomum zeylanicum</i>	Cinnamon	Bark
Labiatae	<i>Origanum marjorana</i>	Sweet Marjoram	Leaves, flowers
	<i>Rosmarinus officinalis</i>	Rosemary	Leaves
Cucurbitaceae	<i>Citrullus colocynthis</i>	Bitter apple	Dried pulp
Urticaceae	<i>Ficus Carica</i>	Common fig	Fruits
Oleaceae	<i>Olea Europaea</i>	Olive	Oil of the fruit
Liliaceae	<i>Allium sativum</i>	Garlic	Bulb
Rutaceae	<i>Citrus limon</i>	Limon	Fruits
Malvaceae	<i>Hibiscus sabdariffa</i>	Roselle	Flowers
Lamiaceae	<i>Mentha piperita</i>	Peppermint	Leaves

Table 2. MIC and MBC (mg/mL) of tested plant extracts against tested organisms

Plant extracts		Tested organisms --- (MIC) /(MBC) (mg/mL)									
		<i>Pseudomonas aeruginosa</i> NCTC 10662	<i>Klebsiella pneumoniae</i> ATCC 10031	<i>Escherichia coli</i> ATCC 25922	<i>Staphylococcus aureus</i> ATCC 25923	<i>Streptococcus pyogenes</i> ATCC 12344	MβL, <i>P. aeruginosa</i>	ESβL, <i>K. pneumoniae</i>	ESβL, <i>E. coli</i>	MRSA	MDR, <i>S. pyogenes</i>
<i>Cassia acutifolia</i>	a	-	-	-	-	-	-	-	-	-	-
	b	100/200	-	-	100/100	-	-	-	-	-	-
	c	-	-	-	-	-	-	-	-	-	-
<i>Nigella sativa</i>	a	100/100	-	50/50	25/25	-	100/100	-	-	-	-
	b	100/100	100/100	25/50	25/50	50/50	100/200	50/100	100/200	25/50	100/200
	c	-	100/200	100/200	25/50	100/200	-	100/200	-	50/50	-
<i>Foeniculum vulgare</i>	a	-	-	6.25/6.25	25/25	-	-	-	-	-	-
	b	100/200	-	6.25/12.5	12.5/25	-	-	-	6.25/12.5	12.5/25	-
	c	-	-	-	25/25	-	-	-	-	25/50	-
<i>Curcuma longa</i>	a	12.5/25	25/50	6.25/12.5	6.25/6.25	25/50	-	-	-	12.5/25	-
	b	6.25/6.25	6.25/6.25	3.13/3.13	12.5/25	25/50	12.5/12.5	-	6.25/6.25	6.25/6.25	25/50
	c	6.25/12.5	25/25	6.25/12.5	12.5/12.5	-	12.5/25	-	6.25/12.5	12.5/25	50/50
<i>Lepidium sativum</i>	a	25/25	-	6.25/6.25	6.25/12.5	-	-	-	-	12.5/25	-
	b	12.5/25	6.25/12.5	3.13/3.13	6.25/6.25	50/50	25/25	-	12.5/12.5	12.5/25	100/50
	c	25/25	12.5/25	6.25/12.5	3.13/6.25	-	25/50	12.5/12.5	12.5/25	12.5/25	-
<i>Punica granatum</i>	a	-	25/50	12.5/12.5	6.25/6.25	-	-	-	-	25/ 50	-
	b	6.25/12.5	12.5/25	12.5/12.5	3.13/6.25	12.5/25	6.25/12.5	25/50	50/100	12.5/12.5	50/50
	c	12.5/12.5	12.5/25	25/25	12.5/12.5	25/25	6.25/12.5	-	50/50	12.5/25	50/50
<i>Foenum graecum</i>	a	-	-	50/100	25/50	-	-	-	-	50/100	-
	b	50/100	-	25/50	-	50/50	100/100	-	50/100	50/50	100/100
	c	50/100	-	50/50	12.5/25	50/100	-	-	-	100/100	50/100
<i>Zingiber officinale</i>	a	50/100	-	-	25/50	-	-	-	-	-	100/100
	b	25/50	100/200	100/200	100/100	100/100	50/50	50/50	-	50/100	100/100
	c	50/100	-	100/200	100/200	100/100	50/100	100/200	-	50/50	-
<i>Anastatica hierochuntica</i>	a	-	-	-	100/100	-	-	-	-	-	-
	b	100/100	100/200	100/100	200/200	100/200	100/200	50/100	50/100	50/50	-
	c	100/100	-	-	50/100	-	-	-	100/100	100/100	-
<i>Capsicum annuum</i>	a	-	-	-	-	-	-	-	-	-	-
	b	-	-	100/200	100/100	200/ >200	-	-	-	-	100/200
	c	-	-	-	-	-	-	-	-	-	-
<i>Cinnamomum zeylanicum</i>	a	100/100	-	-	1.56/3.13	25/50	-	-	-	6.25/12.5	50/100
	b	50/100	100/200	12.5/25	3.13/3.13	12.5/25	100/100	-	-	12.5/12.5	25/25

	c	-	-	6.25/12.5	1.56/3.13	25/25	200/200	-	-	12.5/12.5	-
<i>Origanum marjorana</i>	a	-	-	-	-	-	-	-	-	-	-
	b	-	100/200	100/200	100/100	-	-	-	100/200	100/100	-
	c	-	-	-	100/200	-	-	-	-	200/200	-
<i>Rosmarinus officinalis</i>	a	-	-	-	-	-	-	50/100	-	-	-
	b	50/100	50/50	50/50	12.5/25	100/200	100/100	-	50/50	100/100	-
	c	100/100	-	25/50	50/50	50/100	100/200	-	-	100/200	-
<i>Citrullus colocynthis</i>	a	100/100	50/50	-	50/50	-	-	50/100	-	50/50	-
	b	12.5/25	12.5/25	25/50	-	25/25	-	50/50	25/50	100/100	-
	c	100/200	50/50	50/50	50/100	12.5/12.5	200/200	-	50/100	100/200	25/50
<i>Ficus Carica</i>	a	50/100	50/100	-	50/50	-	-	-	-	-	-
	b	25/25	25/25	100/100	-	200/>200	50/50	100/200	50/50	50/50	-
	c	50/100	25/50	50/50	50/100	-	50/100	-	50/50	50/100	-
<i>Olea Europaea</i>	a	-	-	-	25/50	25/50	-	-	-	-	50/50
	b	100/100	12.5/25	6.25/6.25	12.5/25	12.5/25	200/>200	-	25/50	12.5/25	50/100
	c	-	25/25	12.5/12.5	12.5/25	25/25	100/100	-	12.5/25	25/50	25/50
<i>Allium sativum</i>	a	-	-	-	-	-	-	-	-	-	-
	b	50/50	25/50	6.25/12.5	12.5/12.5	6.25/12.5	50/50	-	25/50	25/25	50/50
	c	-	50/100	12.5/12.5	50/50	25/25	50/100	-	-	25/50	50/50
<i>Citrus limon</i>	a	-	-	-	50/50	-	-	-	-	25/50	-
	b	100/100	50/100	12.5/25	12.5/25	25/50	-	-	100/200	12.5/25	-
	c	-	-	50/50	12.5/25	-	-	-	-	-	-
<i>Hibiscus sabdariffa</i>	a	-	50/100	-	-	50/100	-	-	-	-	-
	b	50/100	25/50	12.5/12.5	12.5/25	12.5/25	-	12.5/25	25/50	50/50	25/50
	c	-	-	25/50	25/25	25/25	-	-	12.5/12.5	25/25	-
<i>Mentha piperita</i>	a	-	-	-	-	-	-	-	-	-	-
	b	100/200	-	12.5/25	6.25/12.5	50/100	-	-	6.25/12.5	50/100	200/>200
	c	-	-	-	25/50	100/100	-	-	-	-	-

- : No antibacterial activity; a: hot water extract; b: ethanol extract; c: acetone extract; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration MDR: multi-drug resistant; ESβL *K. pneumoniae*: Extended Spectrun β Lactamase producing *Klebsiella pneumoniae*; ESβL *E. coli*: Extended Spectrun β Lactamase producing *Escherichia coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *Pseudomonas aeruginosa*; MRSA: Methicillin-resistant *Staphylococcus aureus*.

Table 3. Qualitative chemical analysis of phytoconstituents in different extracts of tested plants.

	Flavonoids.			Alkaloids.			Glycosids			Terpenes			Phenolics			Carbohydrates			Proteins			Saponins			Tannins		
	a	b	C	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	C	a	b	C
<i>Foenum graecum</i>	+	+	-	-	-	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-
<i>Cassia acutifolia</i>	+	+	+	-	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-
<i>Nigella sativa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Foeniculum vulgare</i>	+	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	-	+	+	-	+	+	-	+	+	+
<i>Anastatica hierochuntica</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Lepidium sativum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Punica granatum</i>	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
<i>Curcuma longa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Zingiber officinale</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Capsicum annuum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cinnamomum zeylanicum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Origanum marjorana</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Rosmarinus officinalis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Citrullus colocynthis</i>	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-
<i>Ficus carica</i>	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-
<i>Olea europaea</i>	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+	+	+	-
<i>Allium sativum</i>	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	-	+	-	-	+	-	-	-	+	-
<i>Citrus limon</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
<i>Hibiscus sabdariffa</i>	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+
<i>Mentha piperita</i>	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-

a, hot water extract; b, ethanol extract; c, acetone extract, + : present; - :absent.

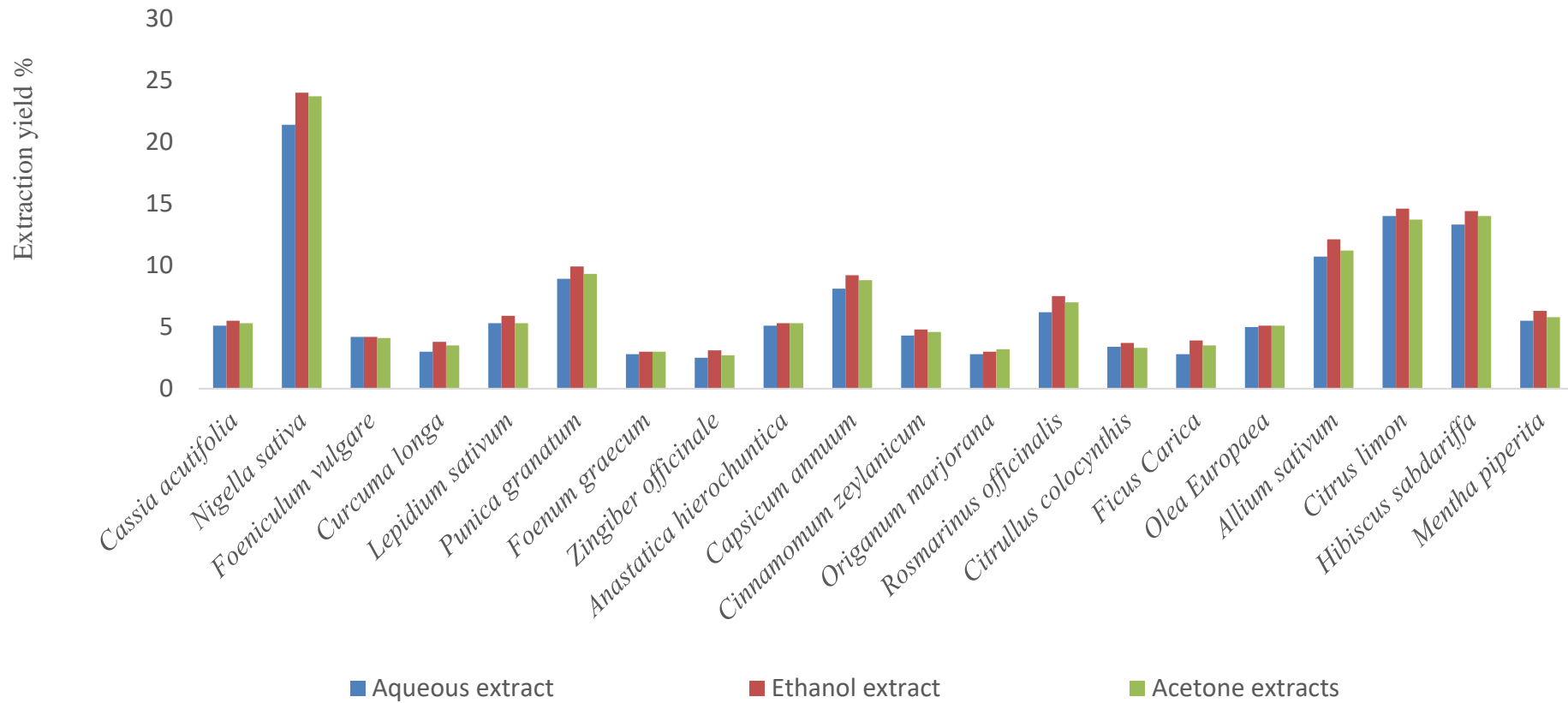


Figure 1: Selected medicinal plants and its extraction yields in %.

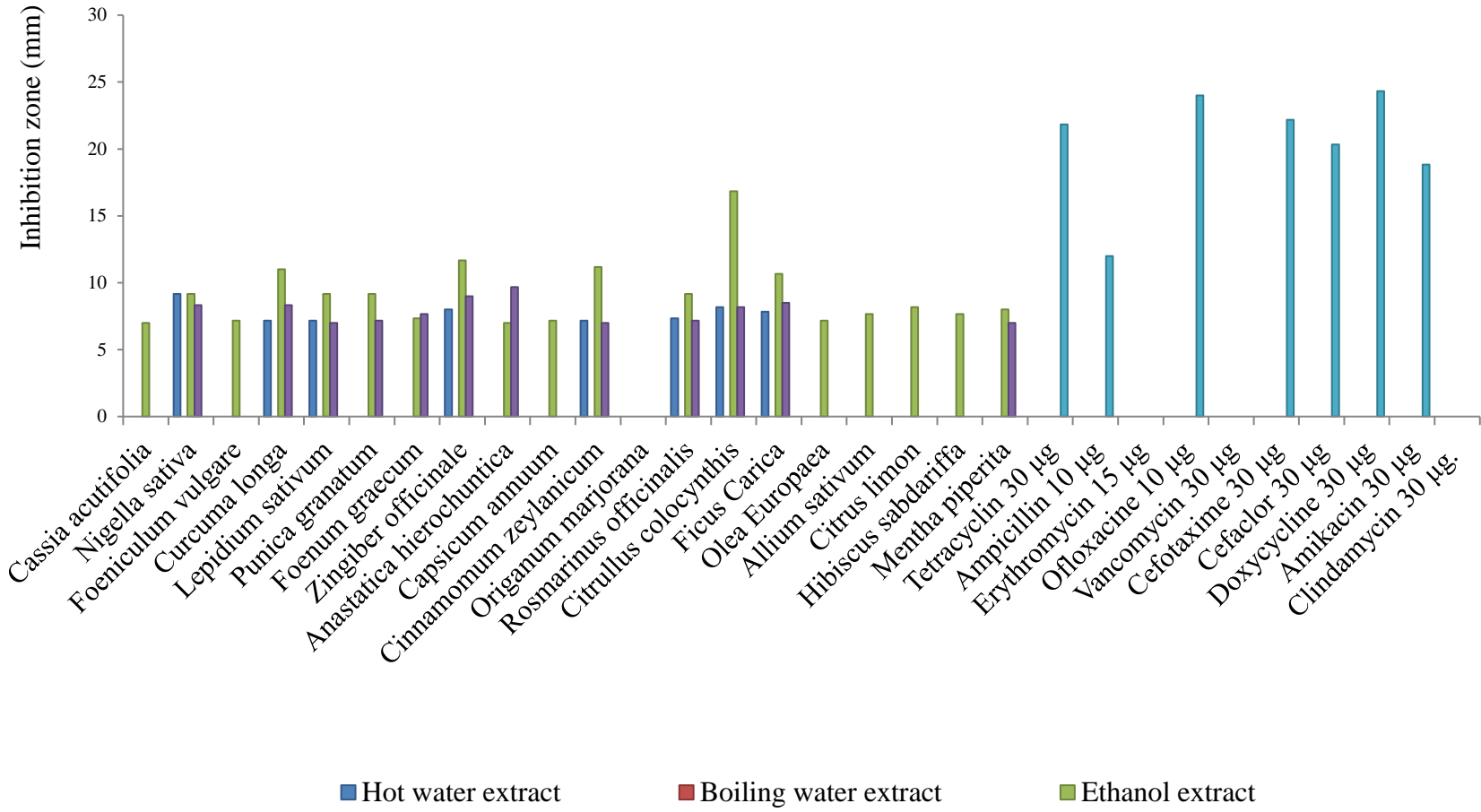


Figure 2: Antimicrobial assay of different plant extracts and stander antibiotics against *P. aeruginosa* (NCTC 10662).

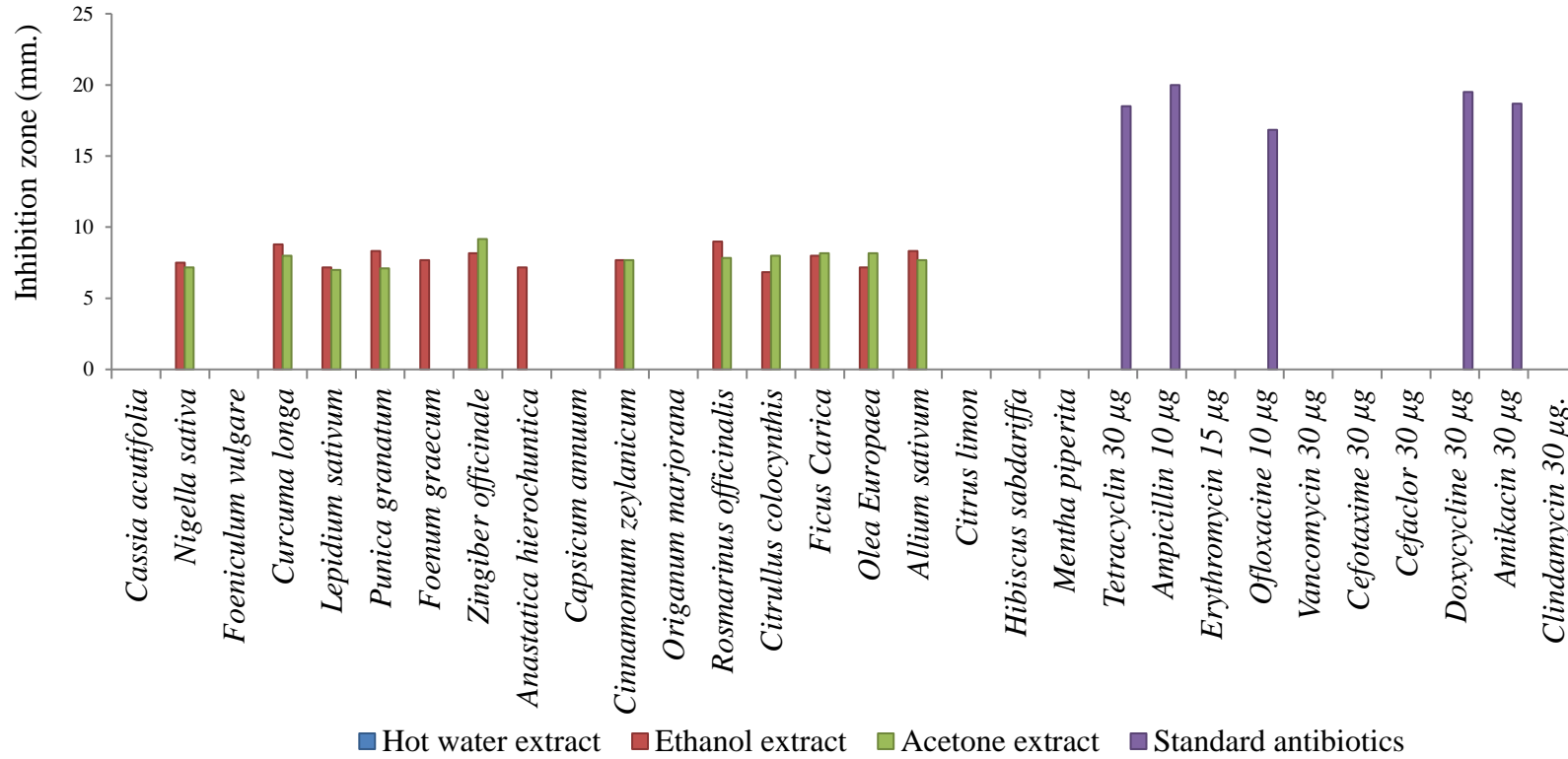


Figure 3: Antimicrobial assay of different plant extracts and standerd antibiotics against MDR *P. aeruginosa*.

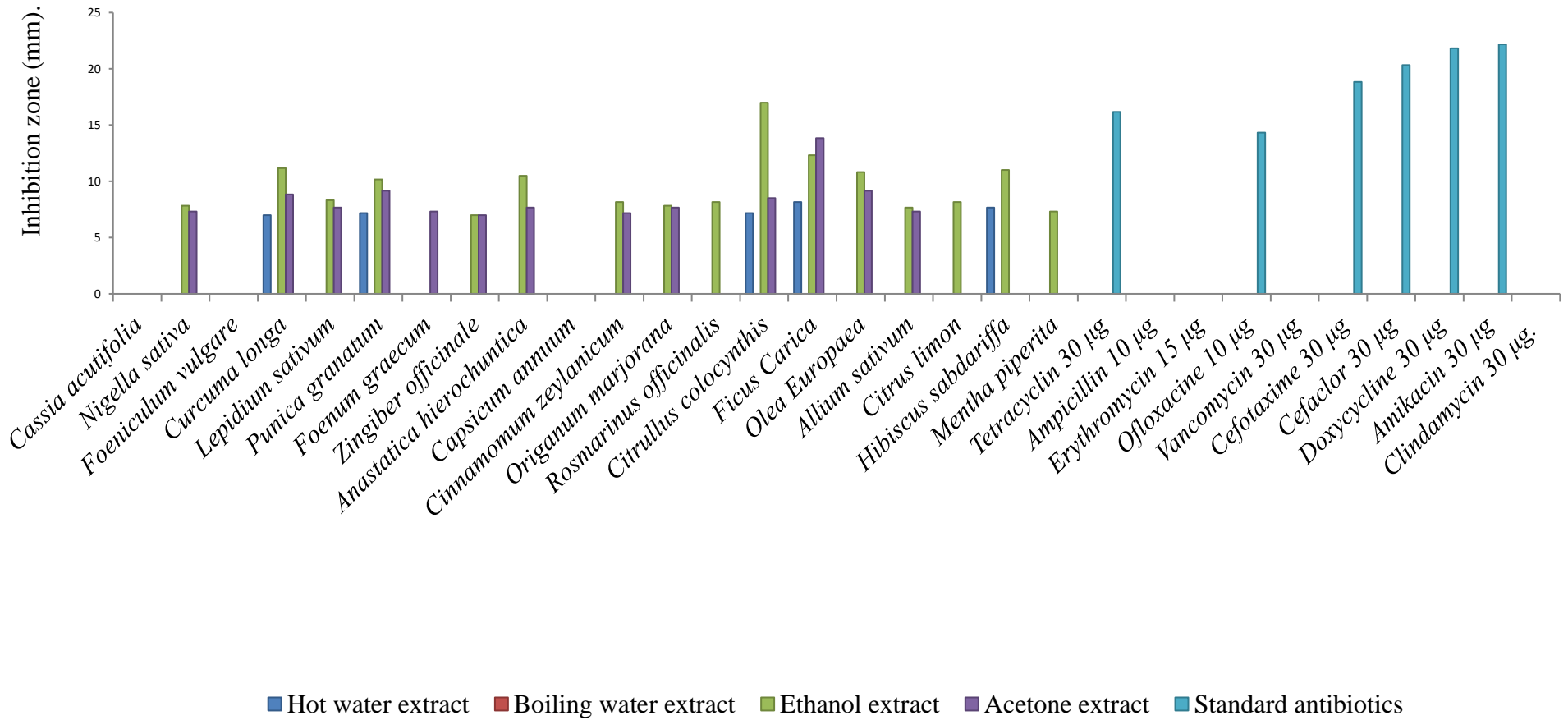


Figure 4: Antimicrobial assay of different plant extracts and standard antibiotics against *K. pneumonia* (ATCC 10031).

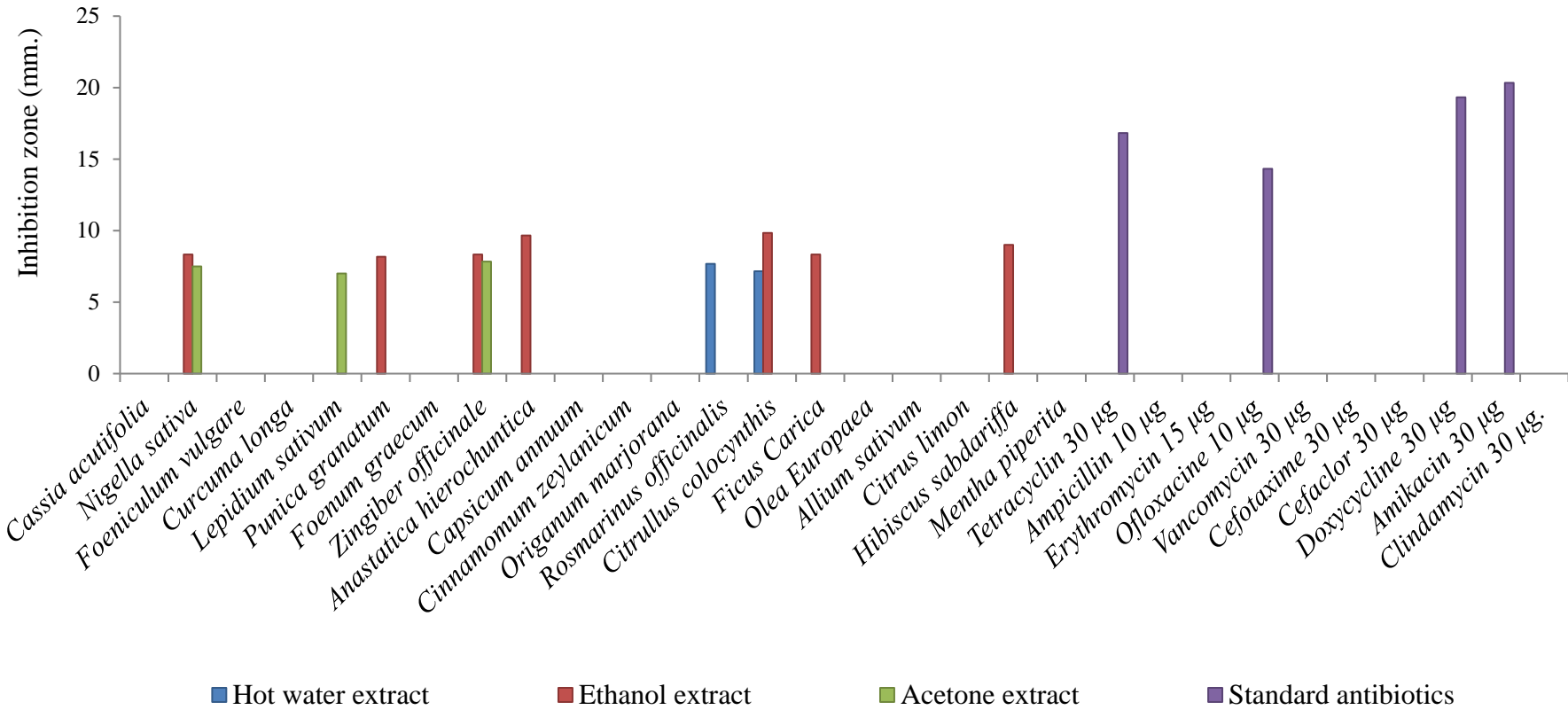


Figure 5: Antimicrobial assay of different plant extracts and standerd antibiotics against ESBL, *K. pneumonia*.

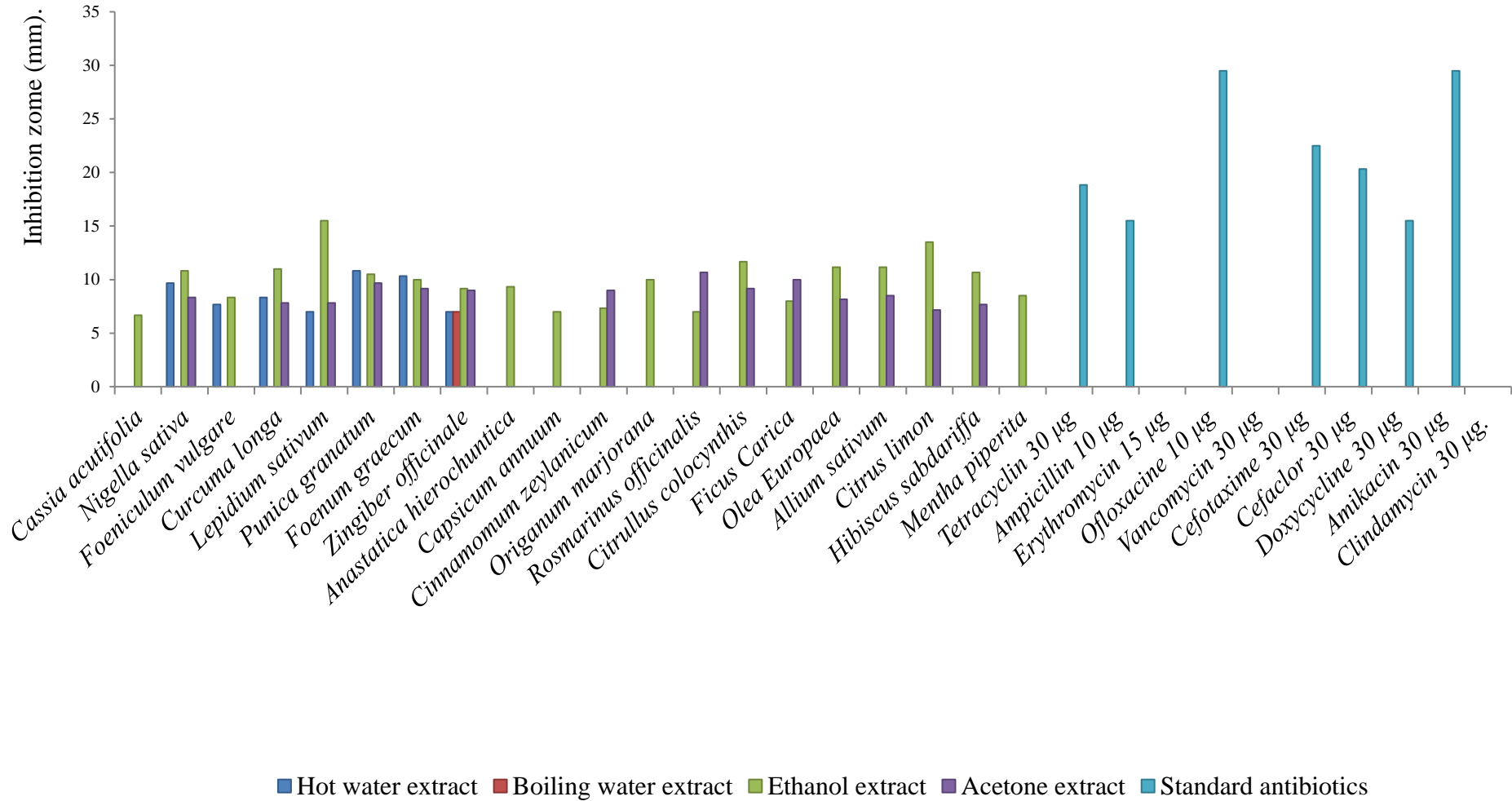


Figure 6: Antimicrobial assay of different plant extracts and standerd antibiotics against *E. coli* (ATCC 25922).

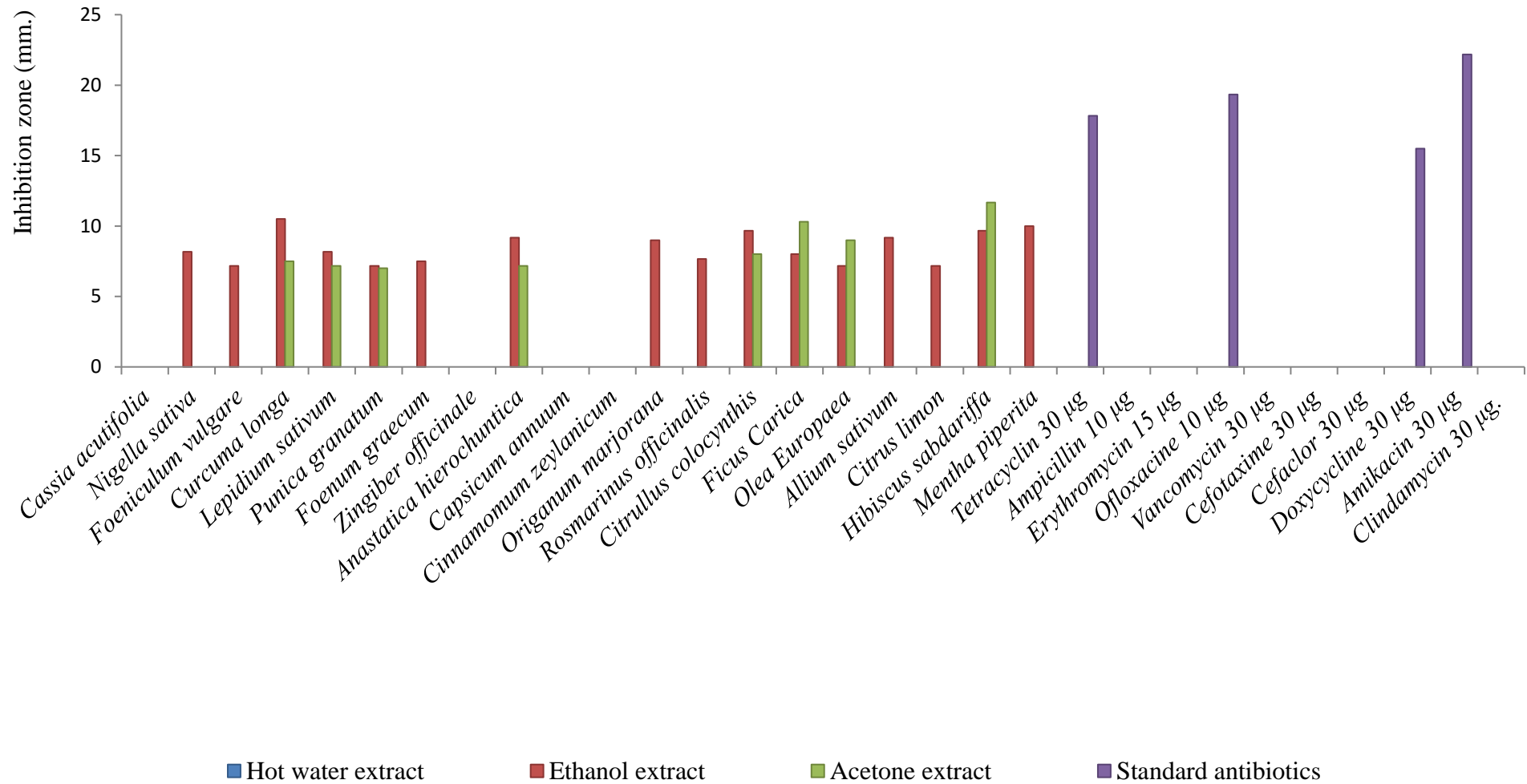


Figure 7: Antimicrobial assay of different plant extracts and standerd antibiotics against ESβL, E.coli.

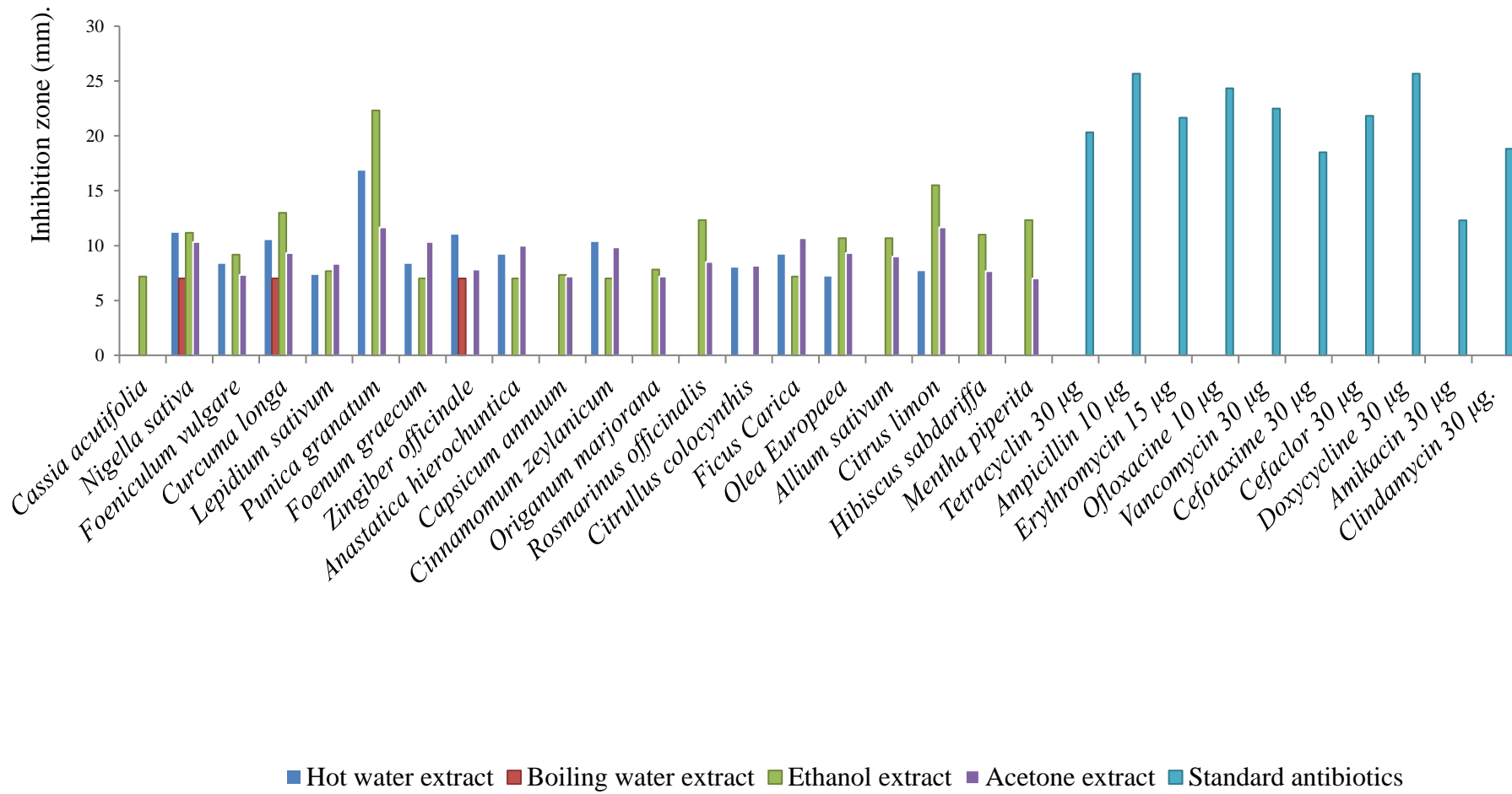


Figure 8: Antimicrobial assay of different plant extracts and standerd antibiotics against *S. aureus* (ATCC 25923).

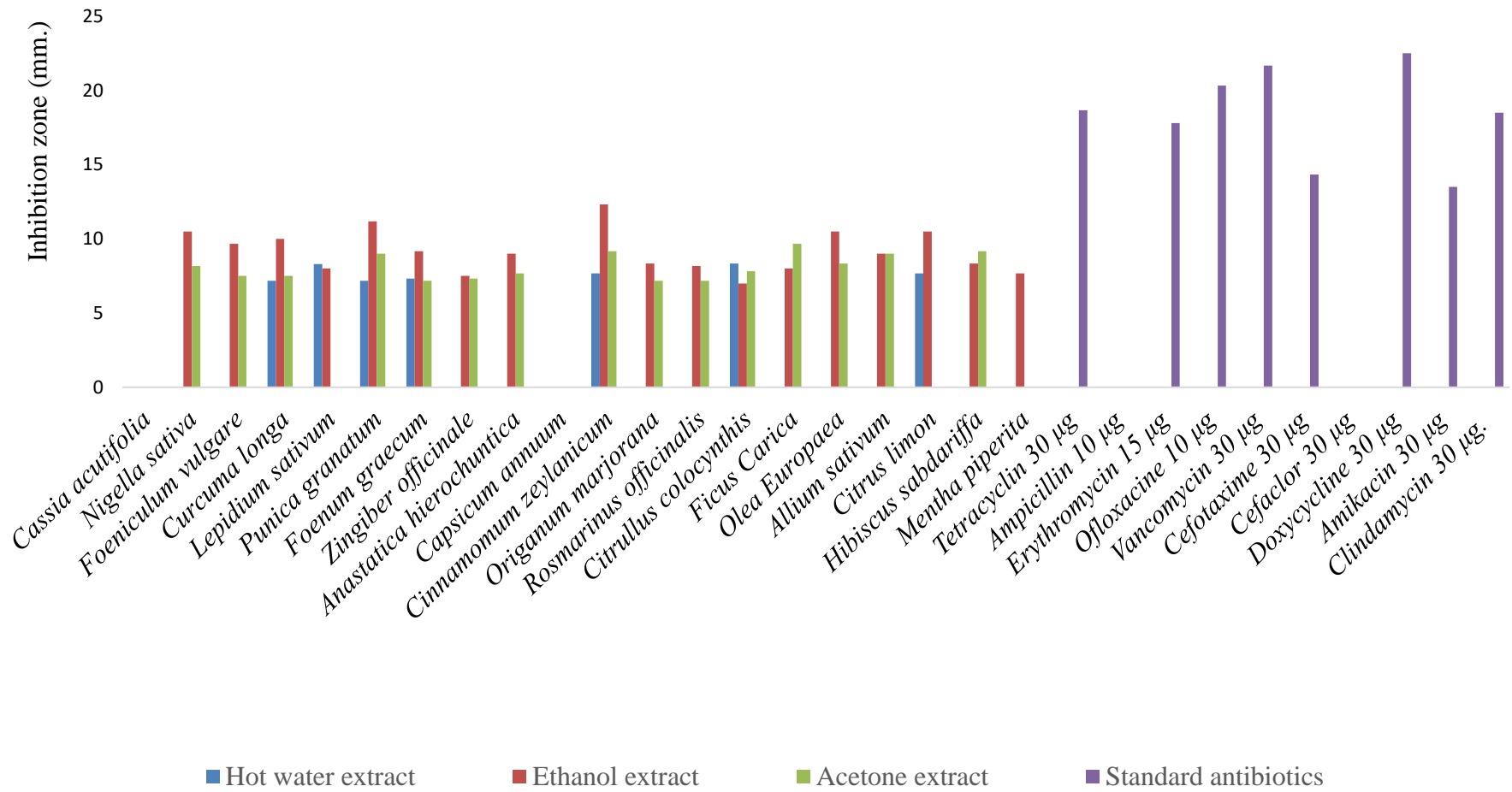


Figure 9: Antimicrobial assay of different plant extracts and standerd antibiotics against MRSA

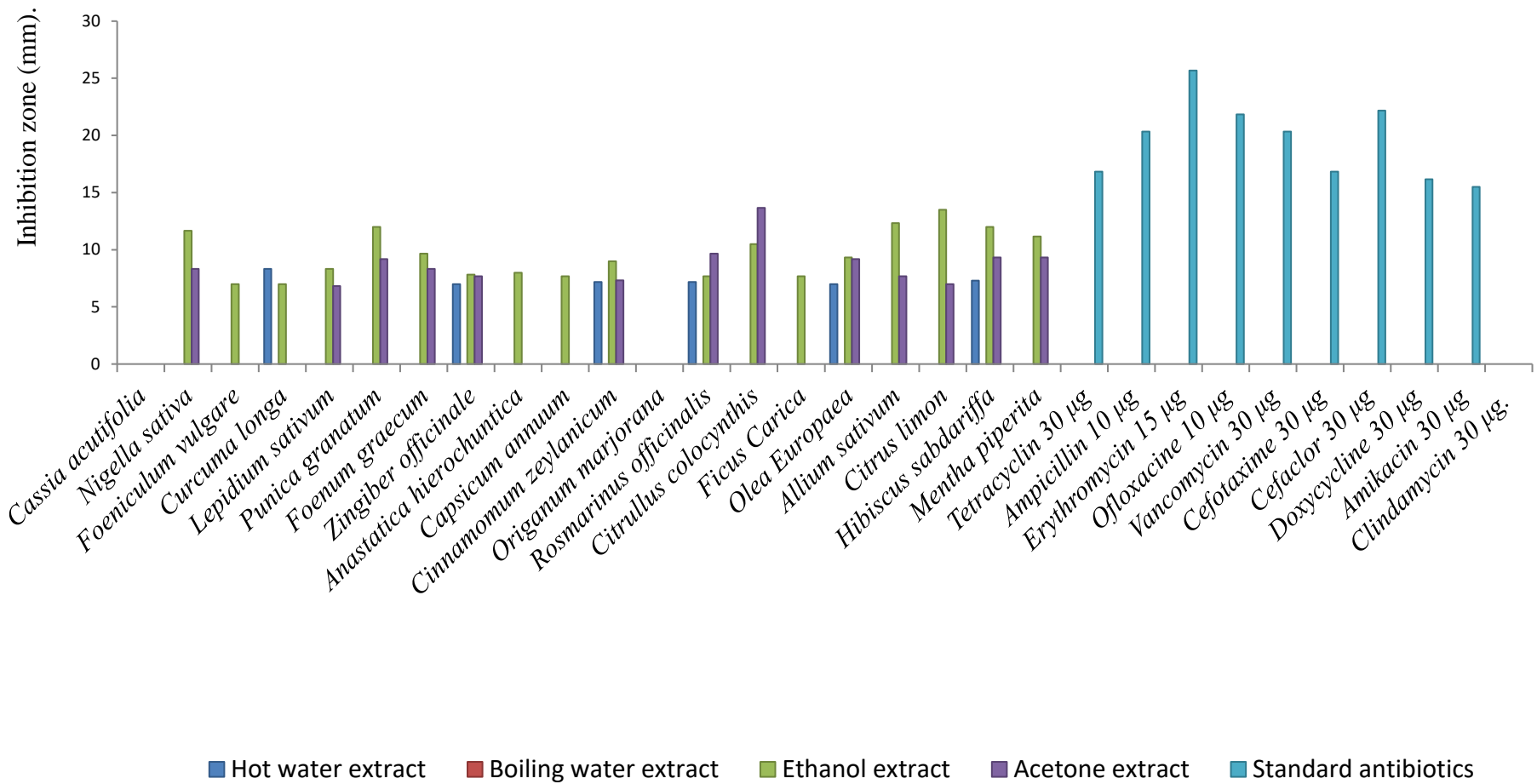


Figure 10: Antimicrobial assay of different plant extracts and standard antibiotics against *S. pyogenes* (ATCC 12344).

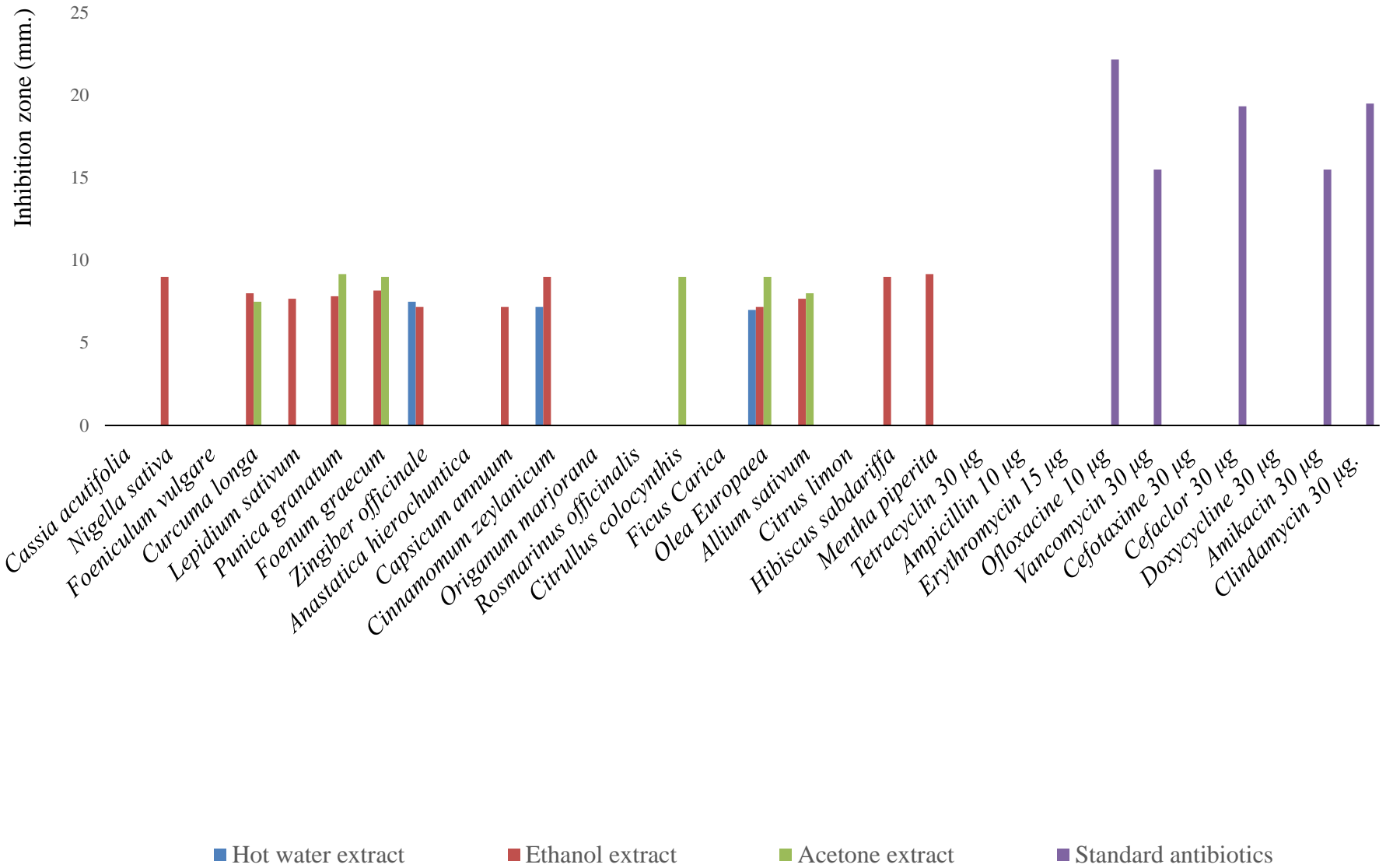


Figure 11: Antimicrobial assay of different plant extracts and standerd antibiotics against MDR *S. pyogenes*.

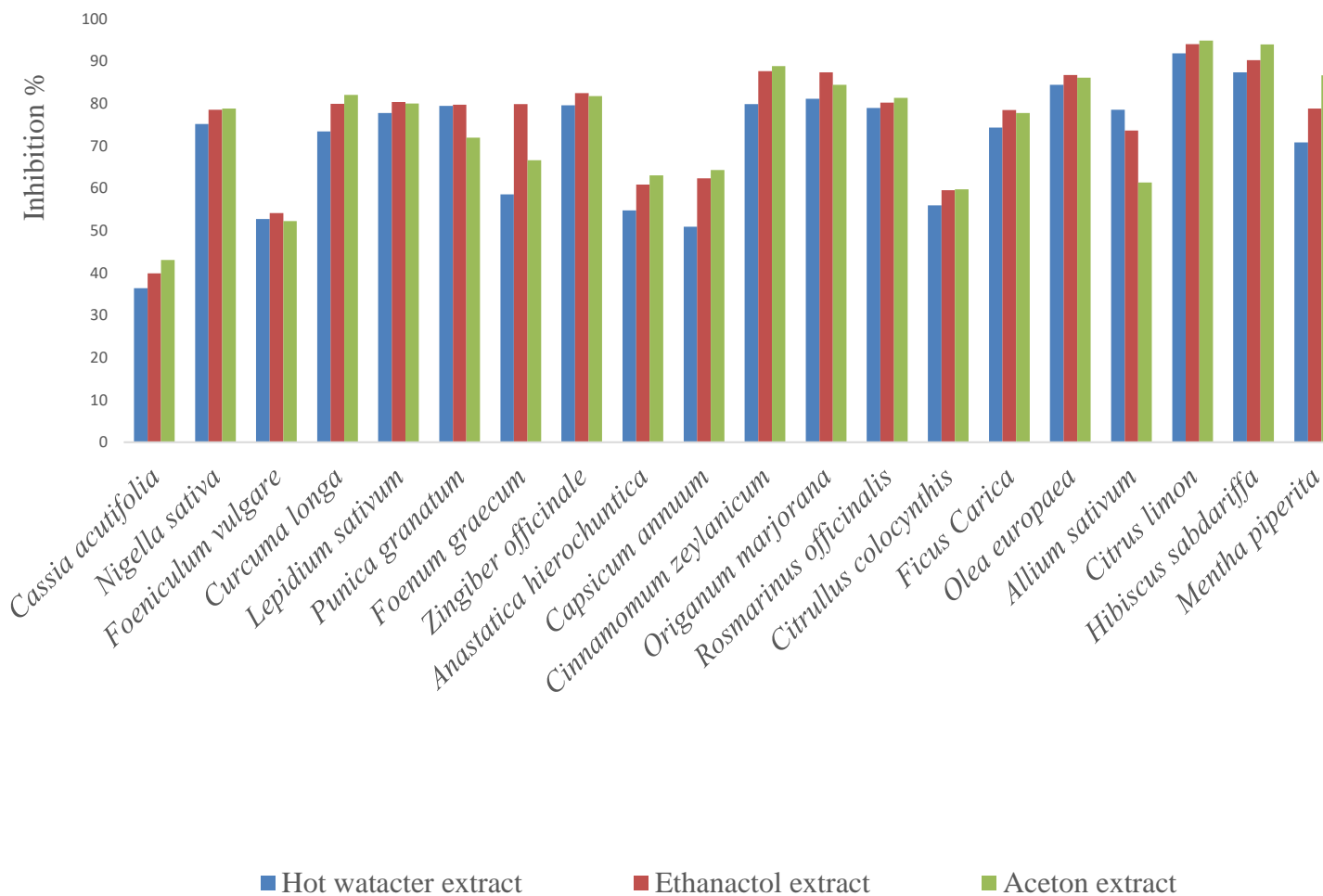


Figure 12. DPPH free radical scavenging activity (% Inhibition) of different extracts of tested medicinal plants.