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, phenoles 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 Egyption folk medicine. The fresh samples were dried at 50ºC, 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 bottels containing soaked plant material were heated in water bath at 40ºC for 2 hrs for hot water extract and for boiling water extract, the bottels were boiled for 30 min. The extracts were allowed to cool for about 6 hrs before it was rapidly filtered through Whattman No. 1 filter paper. For organic extract, 300 gm plant material was percolated with 1L ethanol and 1 L aceton 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 amino-transferase. 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 25ºC 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 aceton 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 annuum*) 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 aceton 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 annuum* and *Cassia acutifolia*.

Aceton 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. Aceton 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, ESβL *E. coli* and *P. aeruginosa* NCTC 10662, and 12.5 mg/mL for MβL, *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 *Foenum 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*, ESβL *E. coli* (3.13 and 6.25 mg/mL, respectively) and NCTC *P. aeruginosa*, MβL, *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*, ESβL *E. coli*, and MβL, *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 (≥ 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 chemo-therapeutic, 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-bacrerial 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 activit. Earlier studies have also shown the anti-bacterial activity of *Foenum graecum* extracts against *Klebsiela 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. pyogens*) 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 ESβL, *E. coli, K. pneumonia*, and *P. aeruginosa*.

Keskin and Toroglu [56] observed the anti-bacterial activities of *Cinnamomum zeylanicum* extracts against *K. pneumonia, 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 ESβL, *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. pneumonae* 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. pyogens, 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, Karuppiah 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. aeuroginosa.* 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, saponnins, flavonoids, etc in *Hibiscus sabdariffa.* Phyto-chemical 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, terpens 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 Złotek *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].

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

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

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

Origanum marjorana	a	-	-	-	-	-	-	-	-	-	-
	b	-	100/200	100/200	100/100	-	-	-	100/200	100/100	-
	c	-	-	-	100/200	-	-	-	-	200/200	-
Rosmarinus officinalis	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	-
Citrullus colocynthis	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
Ficus Carica	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	-
Olea Europaea	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
Allium sativum	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
Citrus limon	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	-	-	-	-	-	-
Hibiscus sabdariffa	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	-
Mentha piperita	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 extrac; b: ethanol extract; c: acetone extract; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration MDR: multi-drug resistant; ESβL *K. pneumonia*: Extended Spectrun β Lactamase producing *Klebsiella pneumonia*; ESβL *E. coli*: Extended Spectrun β Lactamase producing *Escherichia coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *Pseudomonas aeruginosa*; MRSA: Methicillin-resistant *Staphylococcu 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**
Foenum graecum	+	+	-	-	-	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-
Cassia acutifolia	+	+	+	-	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-	+	-
Nigella sativa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Foeniculum vulgare	+	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	-	+	+	-	+	+	-	+	+	+
Anastatica hierochuntica	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lepidium sativum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Punica granatum	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
Curcuma longa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zingiber officinale	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsicum annuum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cinnamomum zeylanicum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Origanum marjorana	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rosmarinus officinalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrullus colocynthis	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-
Ficus carica	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-
Olea europaea	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+	+	+	-
Allium sativum	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	-	+	-	-	+	-	-	-	+	-
Citrus limon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
Hibiscus sabdariffa	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+
Mentha piperita	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-

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

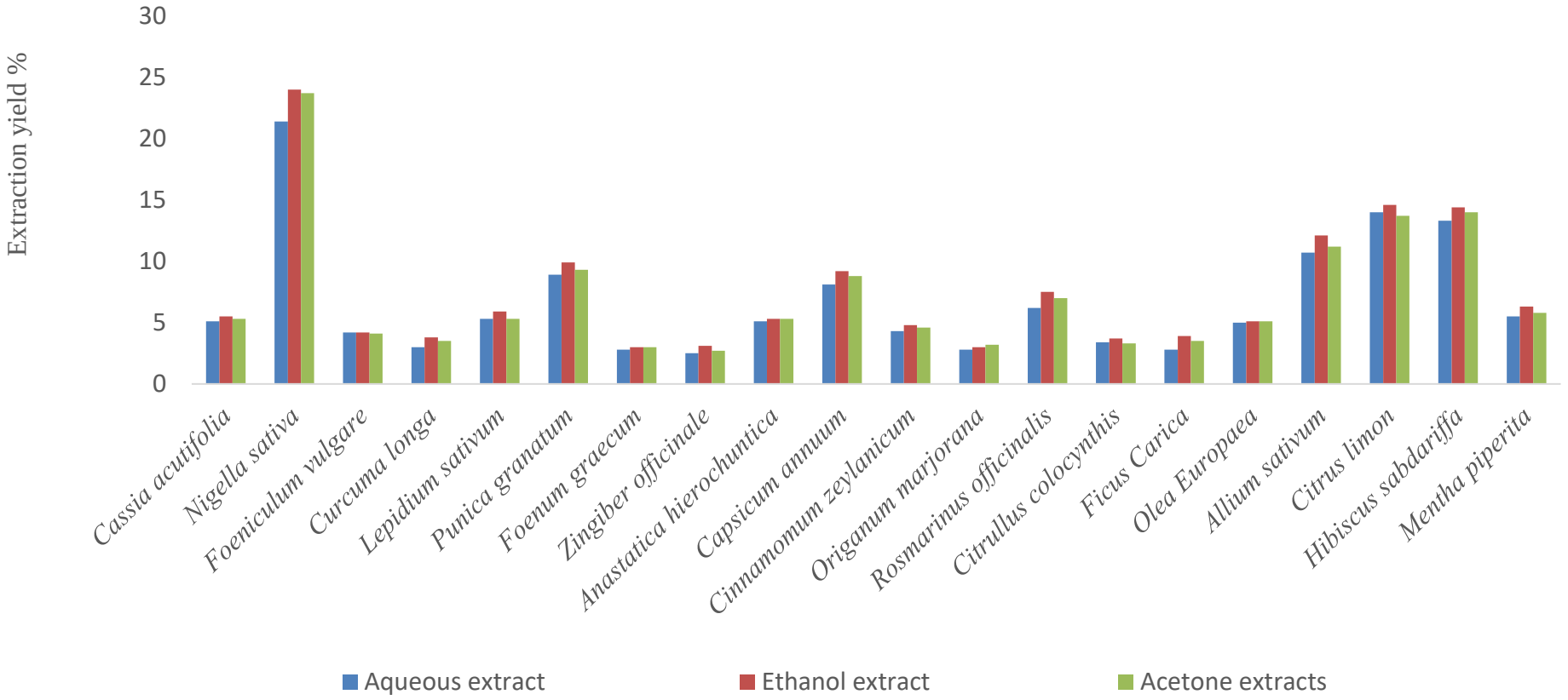

Figure 1: Selected medicinal plants and its extraction yields in %.

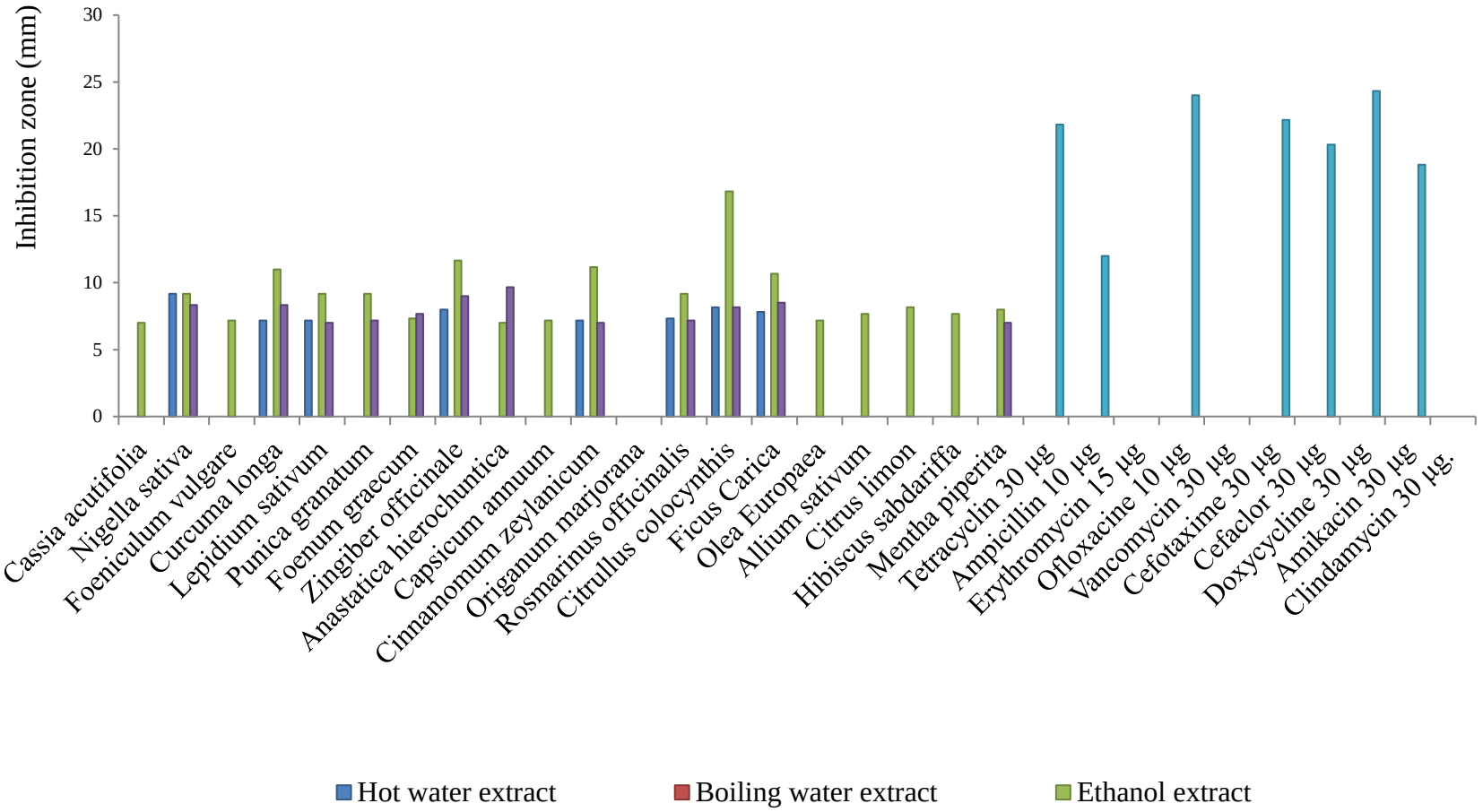

Figure 2: Antimicrobial assay of different plant extracts and standerd antibiotics against *P. aeruginosa* (NCTC 10662).

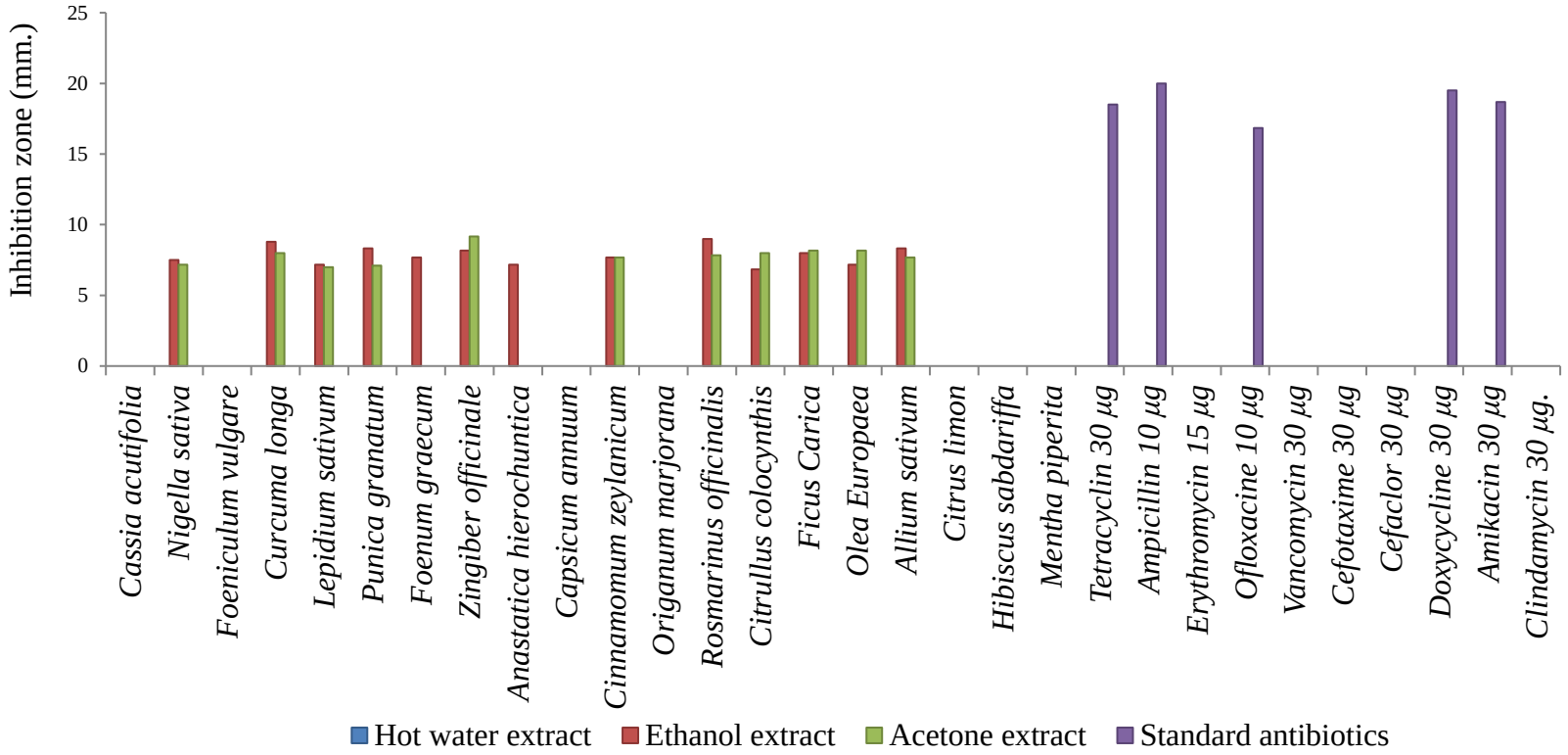

Figure 3: Antimicrobial assay of different plant extracts and standerd antibiotics against MDR *P. aeruginosa.*

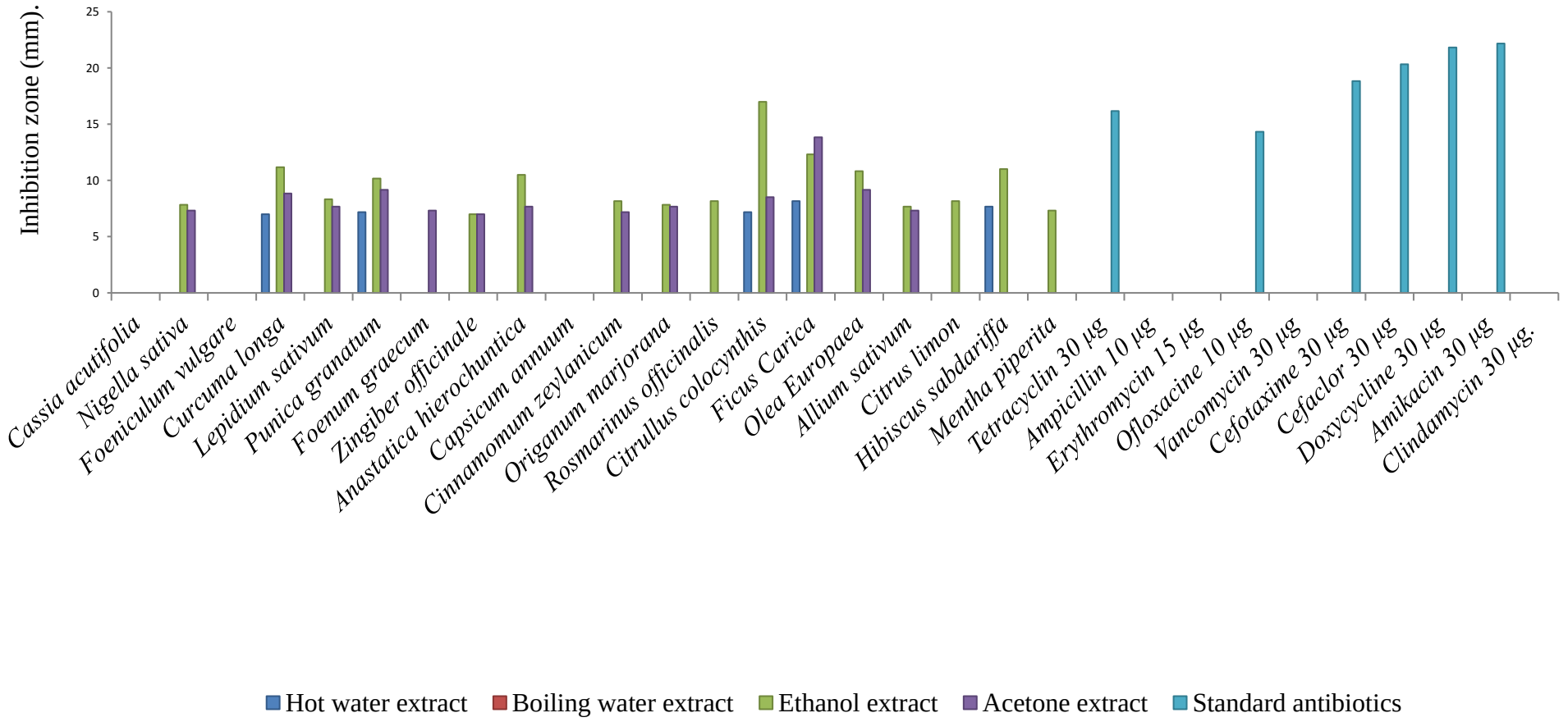

Figure 4: Antimicrobial assay of different plant extracts and standerd antibiotics against *K. pneumonia* (ATCC 10031).

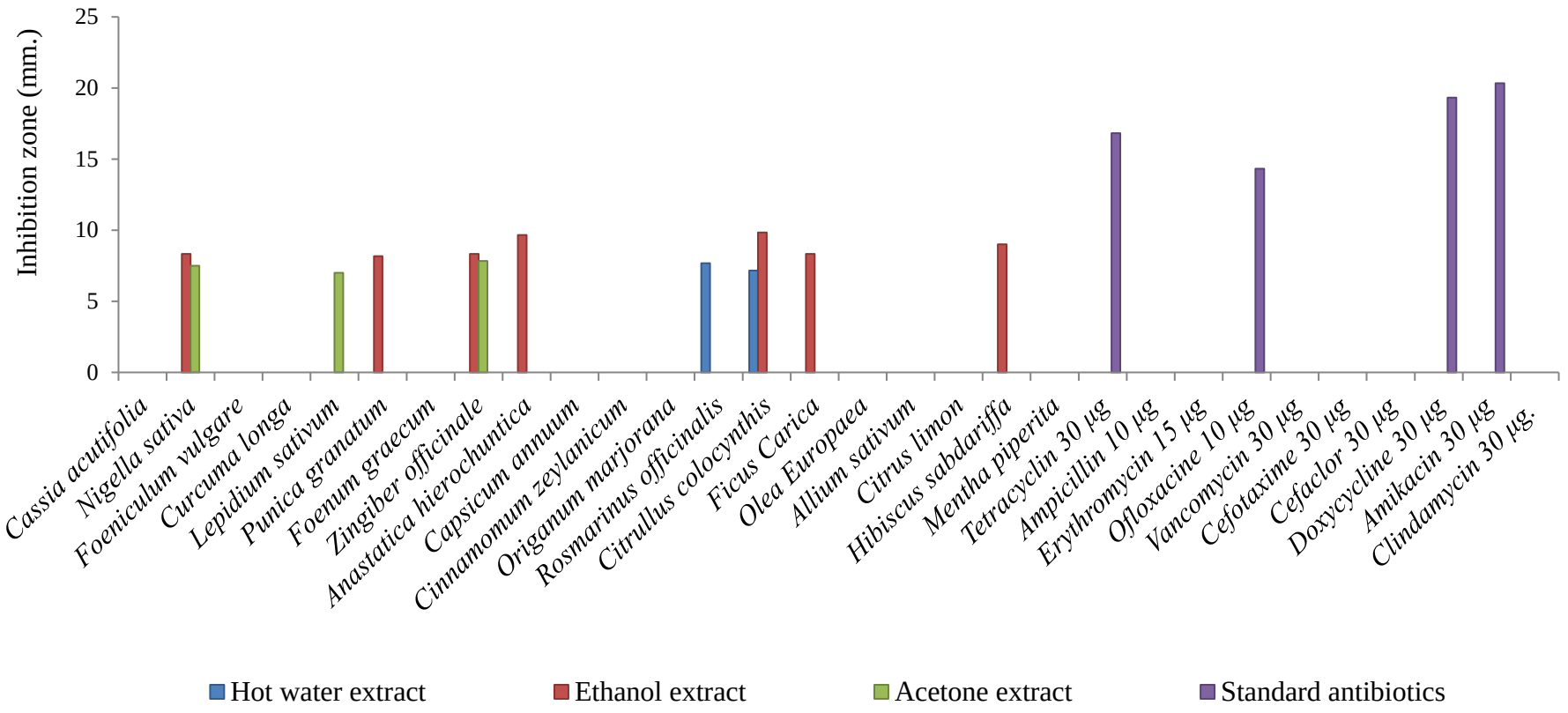

Figure 5: Antimicrobial assay of different plant extracts and standerd antibiotics against ESβL, *K. pneumonia.*

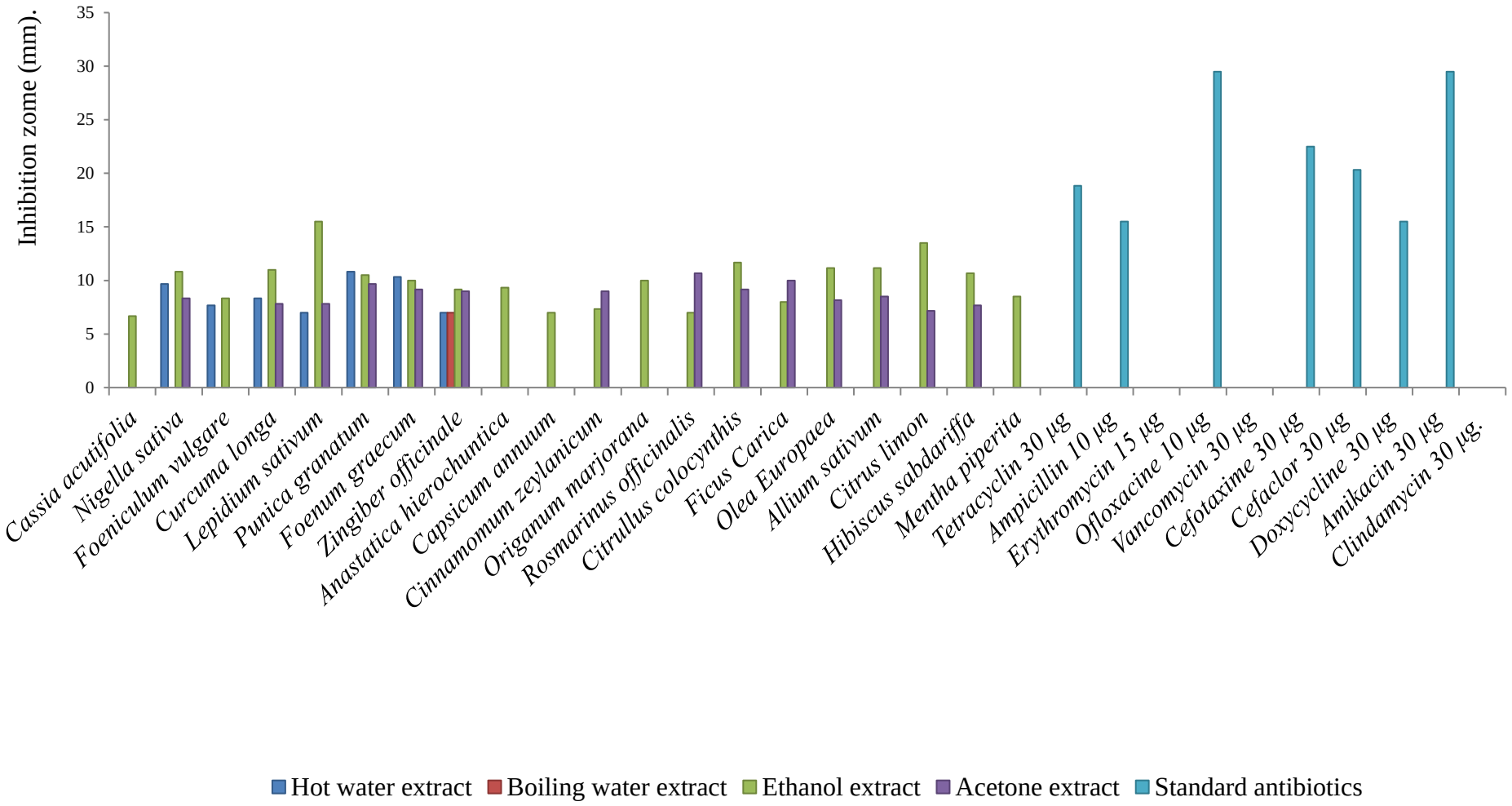

Figure 6: Antimicrobial assay of different plant extracts and standerd antibiotics against *E. coli* (ATCC 25922).

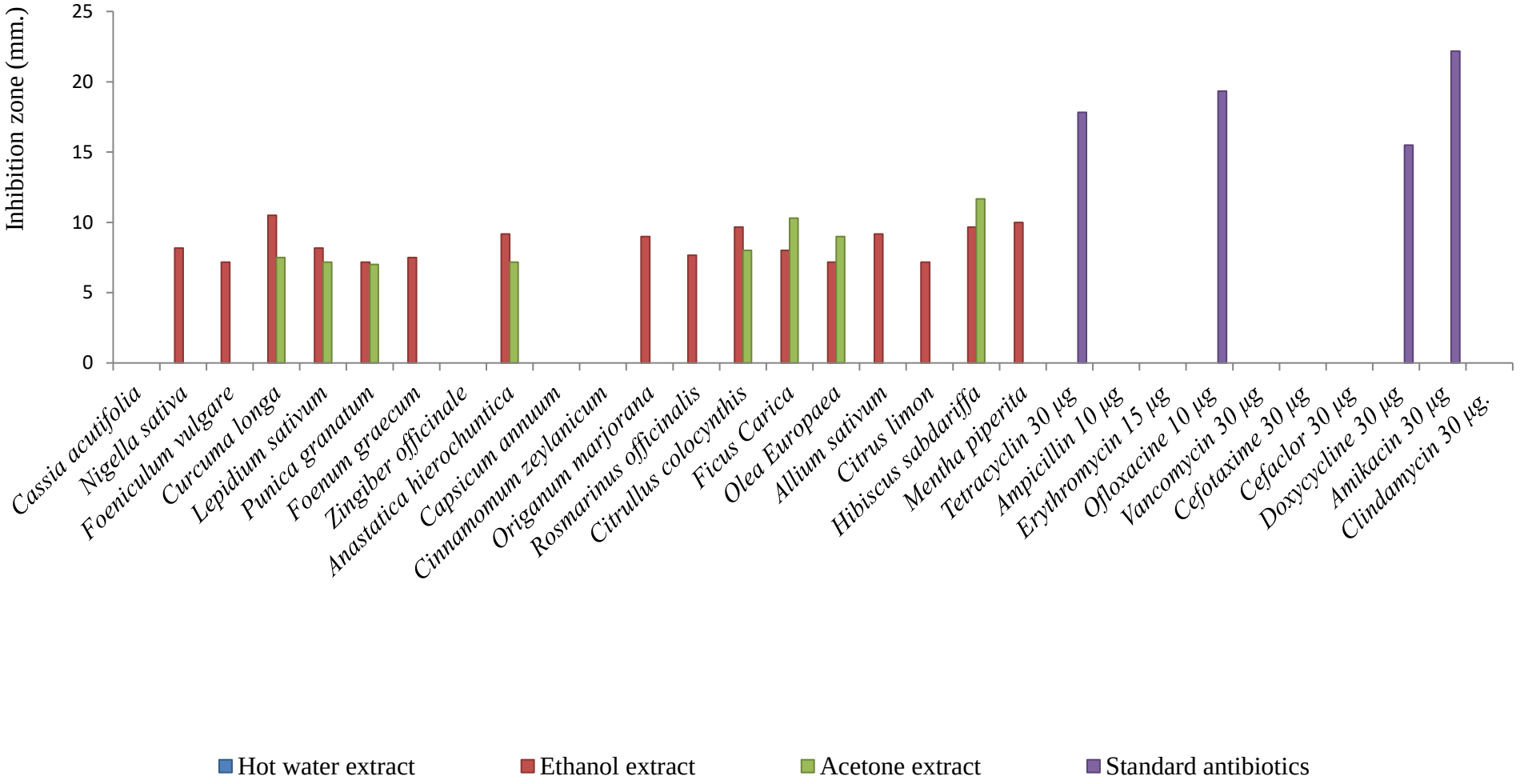

Figure 7: Antimicrobial assay of different plant extracts and standerd antibiotics against ESβL, E.coli.

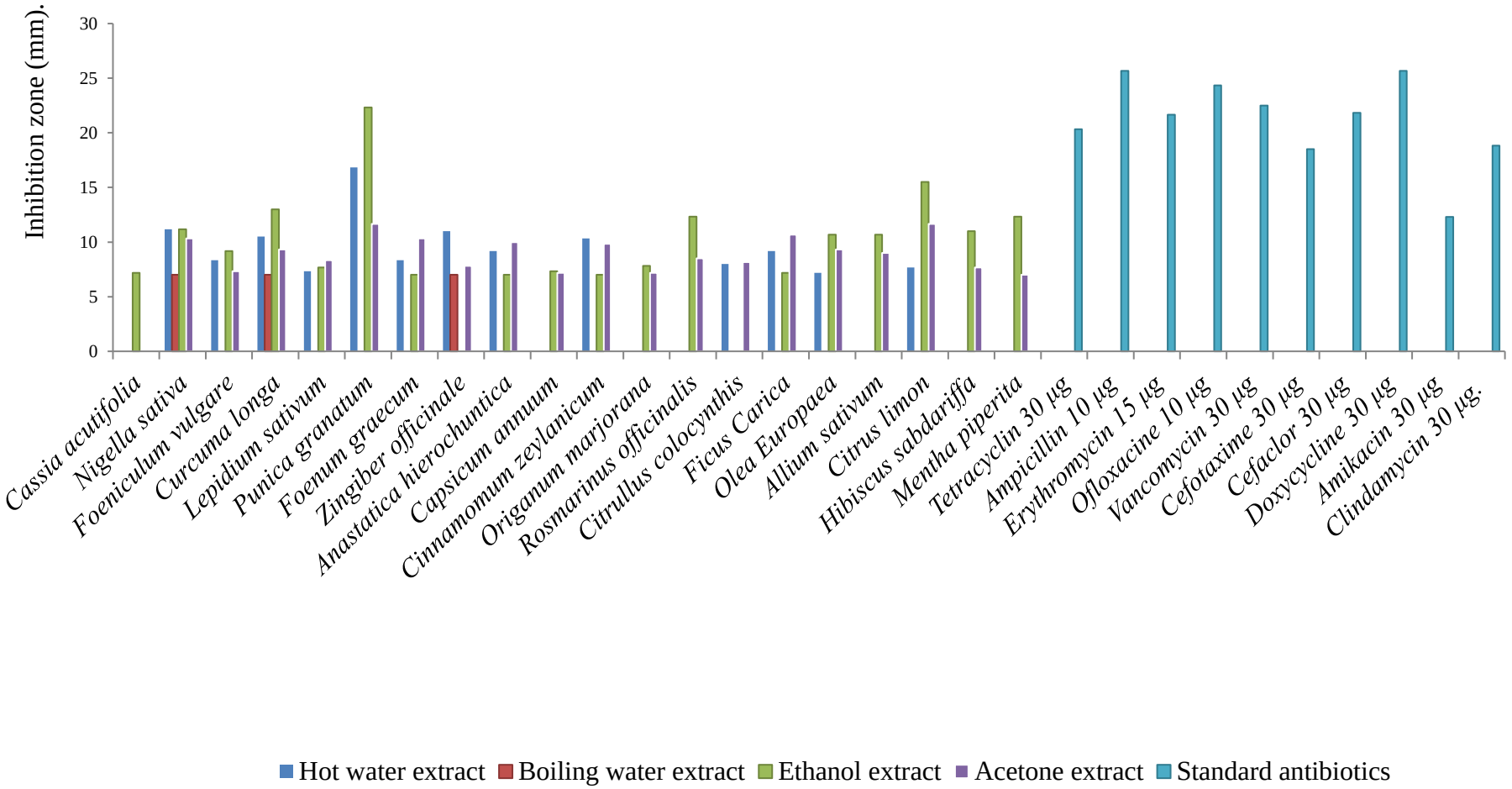

Figure 8: Antimicrobial assay of different plant extracts and standerd antibiotics against *S. aureus* (ATCC 25923).

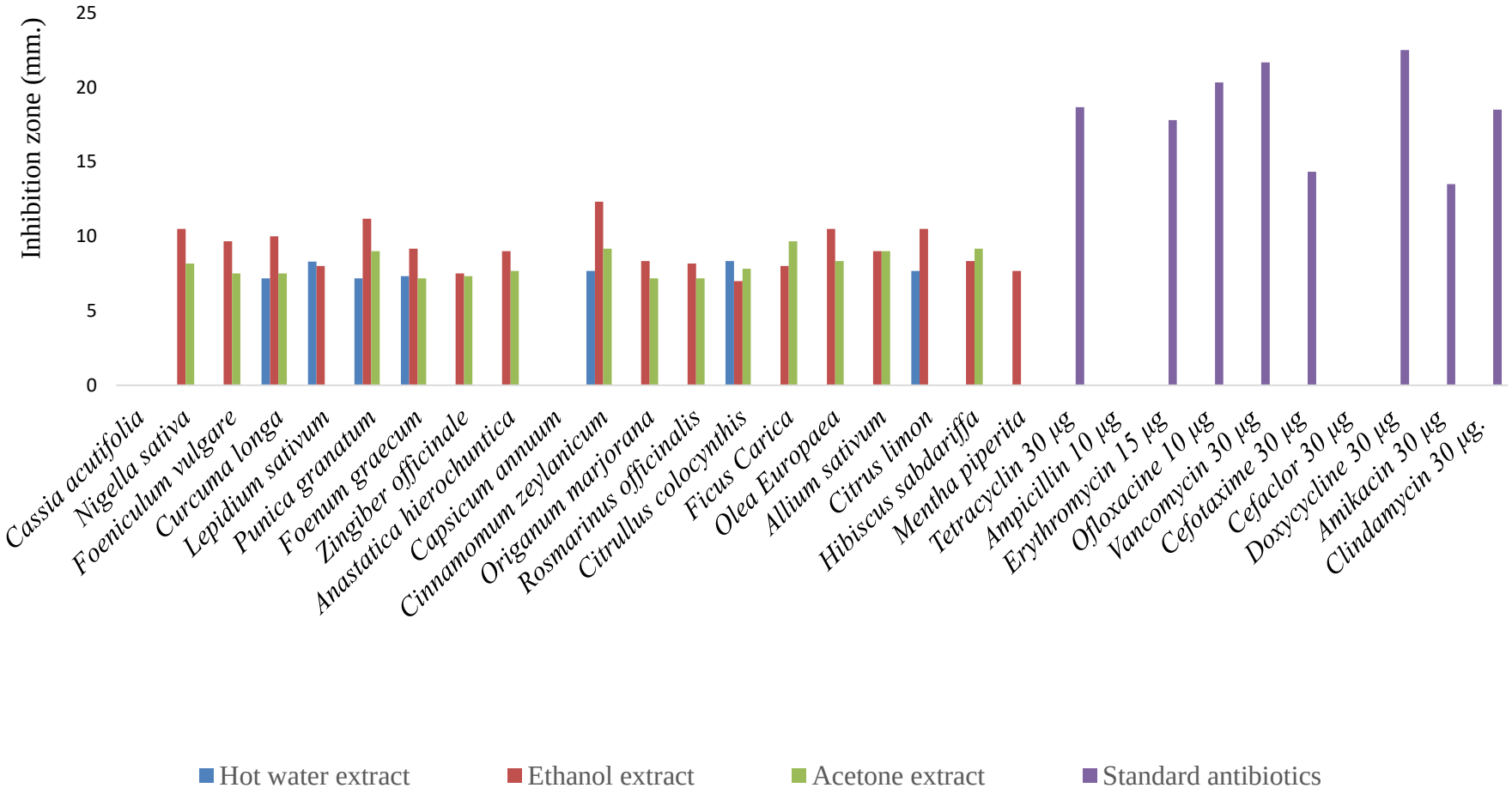

Figure 9: Antimicrobial assay of different plant extracts and standerd antibiotics against MRSA

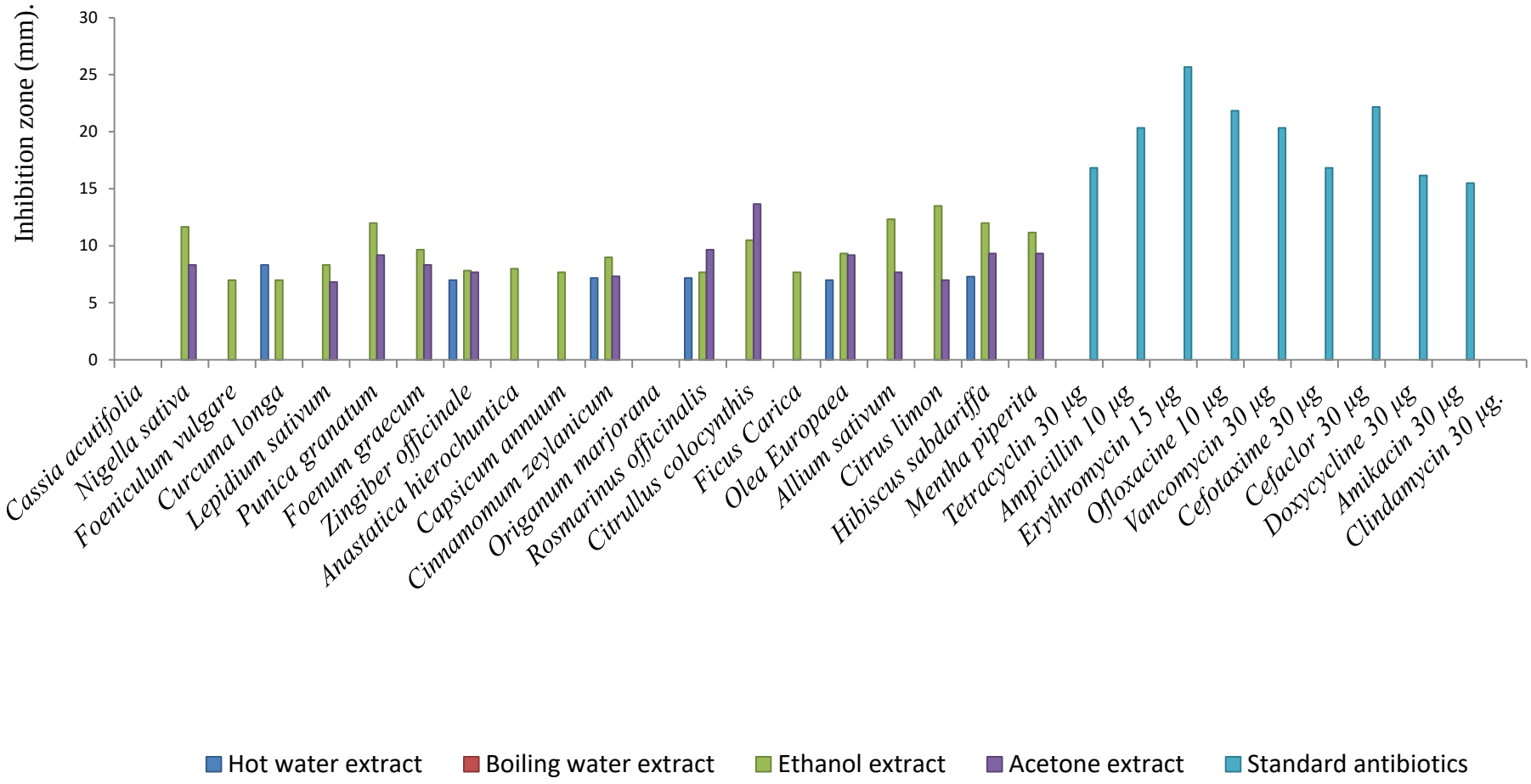

Figure 10: Antimicrobial assay of different plant extracts and standerd antibiotics against S. pyogenes (ATCC 12344).

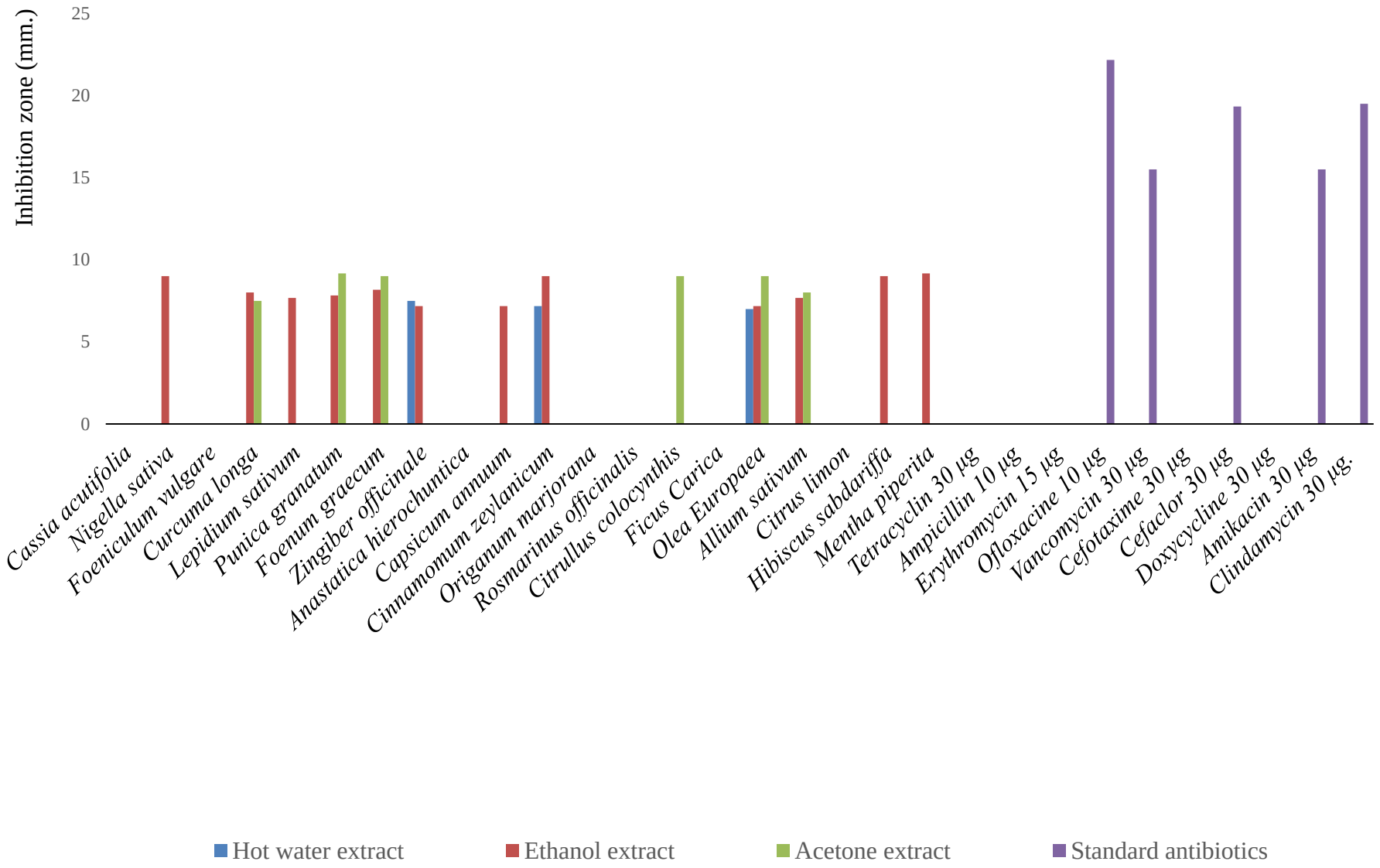

Figure 11: Antimicrobial assay of different plant extracts and standerd antibiotics against MDR *S. pyogenes.*

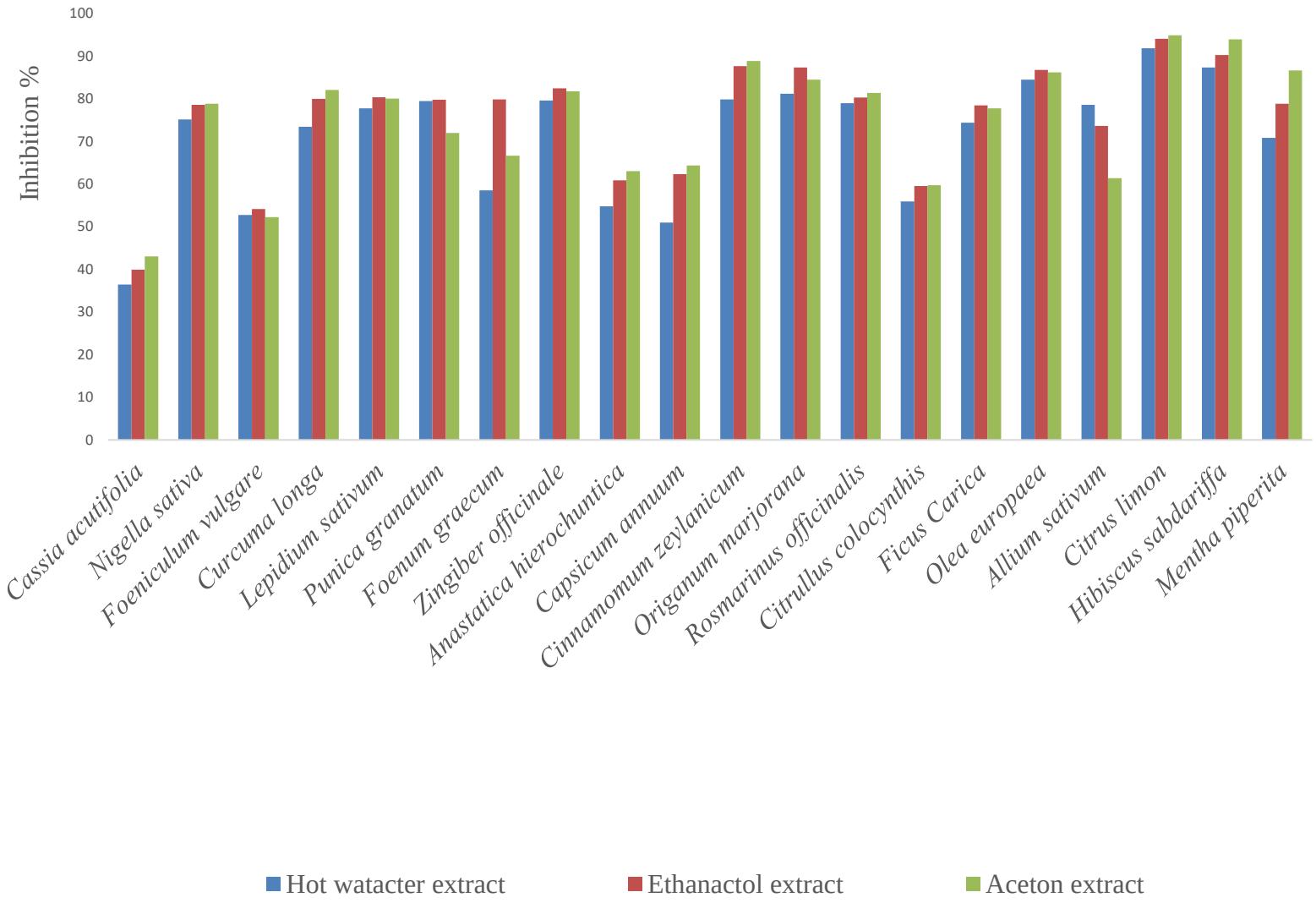

Figure 12. DPPH free radical scavenging activity (% Inhibition) of different extracts of tested medicinal plants.