



## Screening of the antioxidant and antibacterial effects of extracted essential oils from *Thunbergia coccinea*, *Acacia polyacantha*, *Polygonum micrcephallum*, *Abies spectabilis* and *Clerodendrum colebrookianum*

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### ABSTRACT

During the previous few decades, it has been seen that there is a rapid emergence of pathogens resistant to multiple antibiotics. This has now become a global crisis. Some unexplored or less explored plants also provide some antibacterial, bactericidal and antioxidant properties. The antibacterial, bactericidal effects of extracted essential oils (EEOs) of *Thunbergia coccinea*, *Acacia polyacantha*, *Polygonum micrcephallum*, *Abies spectabilis* and *Clerodendrum colebrookianum* was tested in comparison with standard antibiotics. The methods chosen were disc diffusion and deduction of minimum inhibitory concentration (MIC) by microbroth dilution assays of the EEOs against the bacterial strains. The antioxidant activity was found out utilizing DPPH free radical scavenging assay, MDA, Hydrogen peroxide radical inhibition assay and Superoxide radical inhibition assay (O<sub>2</sub><sup>-</sup>). Some commonly used standard antibiotics (metronidazole, amoxicillin, clarithromycin, rifampicin, clindamycin and oxacillin,) were utilized to compare the EEO antibacterial action. *Clerodendrum colebrookianum* (85.17 ± 3.06 µg MDA/g extract) had a reasonable MDA. *Acacia polyacantha* in MIC had values of 3.86 ± 0.25 to 6.20 ± 0.16. *Polygonum micrcephallum* had excessive H<sub>2</sub>O<sub>2</sub> (48.27 ± 2.4 5%). The antibacterial actions determined by the paper disc-diffusion technique of the EEO extracted from these plants showed that most had some antibacterial actions. Also, it was seen that the bactericidal action of the EEO extracted from *E. alba* was most potent against *S. pyogenes* (4.06 ± 0.15). The extract of the plant at varying concentrations (20, 40, 60, 80 and 100 mg/mL) demonstrated noteworthy ( $P < 0.001$ ) anthelmintic action in an effective change when the dose was adjusted. In conclusion, most of the tested plants contain a medicinal value, which can be utilized in the future to supplement artificial medicines and cure emerging diseases that create havoc for mankind.

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### Introduction

The majority of the plants utilized for medicinal value are indigenous and unknown to the world of phytochemicals. The vital constituents and efficacious phytochemicals with propitious pharmacological properties available in those plants are still to be reconnoitred (1, 2). It is not rare that some life-restoring marvel drug formulation can come to view from within

one or some of those indigenous plants which are utilized by the local people with confidence by local people and the tribals residing there and can bring about a landmark in pharmaceutical sciences which can become a blessing to humanity all over the world (3). Various factors result in free radicals in our bodies; which give rise to different dysfunctions such as Alzheimer's; liver; cardiovascular; diabetes;

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atherosclerosis; and joint diseases. But our (human) body has developed means of purifying ourselves from these free radicals by virtue of some warriors like glutathione peroxidase; catalase; and superoxide dismutase (4, 5). It has been seen that the majority of wild comestible plant products have a therapeutic property and can be utilized to heal customary afflictions; these are effortlessly accessible; inexpensive and also outstanding originators of nutrients like essential minerals; proteins; iron; other secondary metabolites and carbohydrates. Systematic utilization of these plant products produces an unconventional origin of medicines (6, 7). Therapeutic plants depict a limitless provenance of -saving medicines for most of the world's population (8). The lucrative therapeutic and rehabilitative ramifications of plant substances are predominantly due to the presence of materials called secondary metabolites of plants (9). *Acacia polyacantha*; also known by the name of Samaidh in the Assamese language; is a deciduous tree whose utilization is mainly in folk medicine; for the treatment of various diseases. *Thunbergiacoccinea* is a category of woody climber having a lengthy intense pair of green leaf which commonly supports scarlet-colored flowers during the winter and continues till spring. It is common to name is Changanota; Nilakontho or Nil-lata. It is mainly utilized for the treatment of sterility and infections of the stomach in folk medicine (3, 10, 11). *Abies spectabilis* is a coniferous tree; which is also said to have medicinal values in herbal medicines. *Polygonum micrperhallum* is known in Assamese as Modhuxleng. It is an edible perennial shrub that is said to have many medicinal properties. *Clerodendrum colebrookianum* is also a very much utilized medicinal plant used by seen that there is a rapid emergence of pathogens resistant to multiple antibiotics. This has now become a global crisis (12).

Some unexplored or less explored plants also provide some antibacterial; bactericidal and antioxidant properties. Therefore; we wanted to explore the antibacterial; bactericidal effects of extracted essential oils (EEOs) of *Thunbergia coccinea*; *Acacia polyacantha*; *Polygonum micrperhallum*; *Abies spectabilis* and *Clerodendrum colebrookianum* to find out if these plants can benefit mankind.

## Materials and methods

### Preparation of plant extracts

Extracts from the six plants described above were utilized in the study. Extraction was done as per standard methods (13). A grinding machine was utilized to powder the plant extracts, and then ultrapure sterile water and the ground plant extracts were mixed to prepare a 5% liquid extract by use of heat (at 75°C) for 4 minutes in two successive cycles. Solution filtration was done with the help of a 0.24 µm membrane (14). 95% of methanol was utilized to prepare a plant extract in the ratio of 1:10 (w/v) for 3 days at a temperature of 24°C. Eventually; evaporation of methanol under specific conditions of vacuumed pressure was concluded for the plant extracts and ensuring leftover substances were utilized as the main methanolic extract. DMSO (from Merck; Darmstadt; Germany) was used to prepare stock solutions; and ultimately the final working volumes were attained by regular specific dilution of the primary stock with Mueller-Hinton (MH) broth (from Oxoid; Hampshire; UK).

### DPPH free radical scavenging assay

The measurement of DPPH radical scavenging activity was carried out according to the method of Barros et al. (2007) (15). The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the following equation (Eq. 1):

$$\text{DPPH radical scavenging \%} = ((A_0 - A_1)/A_0) \times 100 \quad (\text{Eq. 1}),$$

Where A<sub>0</sub> is the absorbance of the DPPH solution and A<sub>1</sub> is the absorbance of the sample.

### Quantity of malondialdehyde (MDA)

The amount of MDA was measured according to the combined method invented by Chawla et al. in 1976 (16). This was performed by mixing 0.1 ml of plant extract with 1.9 ml distilled water; 2 ml of trichloroacetic acid; and 2 ml of thiobarbituric acid solution. Then the mixture was subjected to 100°C for 10 minutes in a boiling water bath. Then it was cooled and centrifuged at a speed of 3000 rpm for 20 minutes. After taking it out; the absorbance of the supernatant

was measured at 532 nm. The amount of MDA was enunciated as  $\mu\text{g MDA/g}$  plant extract.

### Hydrogen peroxide radical inhibition assay ( $\text{H}_2\text{O}_2$ )

Determination of  $\text{H}_2\text{O}_2$  scavenging potential of the plant extracts was done by the method delineated by Ruchet et al. in 1989 (17). The following formula was utilized:

Hydrogen peroxide radical scavenging percentage =  $(A \text{ of Blank} - A \text{ of Sample}) / A \text{ of Blank} \times 100$

### Superoxide radical inhibition assay ( $\text{O}_2^-$ )

Scavenging activity (of plant oil) of  $\text{O}_2^-$  radical as delineated by Jing et al. 1995 (18) was utilized. Here; 1 ml of plant extract was mixed with 9 ml of 5 mM Tris-HCl buffer (pH 8.2). Then 40  $\mu\text{l}$  of pyrogallol (4.5 mM) was added to it. Then it was shaken and after 3 minutes; one drop of ascorbic acid (0.035%) was mixed with it. Then the measurement of the absorbance of the mixture was done at a wavelength of 420 nm after a waiting period of 5 minutes. In order to eliminate interference; a similar concentration of the plant extract was utilized as the blank (19). The scavenging effect of the plant extract was finally calculated utilizing the following equation (in percentage):

Superoxide radical scavenging percentage =  $(A_0 - (A_1 - A_2)) / A_0 \times 100$

Where;  $A_0$  = absorbance of the pyrogallol with Tris-HCl buffer;  $A_1$  = absorbance of the added extract; and  $A_2$  = absorbance of blank plant extract.

### Minimum inhibitory concentration determination

Minimum inhibitory concentrations (MICs) ascertainment was done by micro broth dilution assays which utilized MH broth (20). This type of assays of micro broth dilution to define and determine the MICs of the implicated EEO deracinated from different types of plants versus the various bacterial strains. The range of EEO concentrations used in the study for MICs stretched from 5000  $\mu\text{g/mL}$  to 50  $\mu\text{g/mL}$ . Loading of the two-fold dilutions of culture containing 100  $\mu\text{L}$  of extracts; containing each particular strain was done in a flat-bottom 96-well plate; duplicated wells made of polystyrene. Starting inoculum for each strain was  $1.5 \times 10^5$  CFU/mL; and the wells which had bacterial inoculum only i.e.; no compound was presently provided as the controls. Afterward, the experimented plates were put for incubation. The minutest

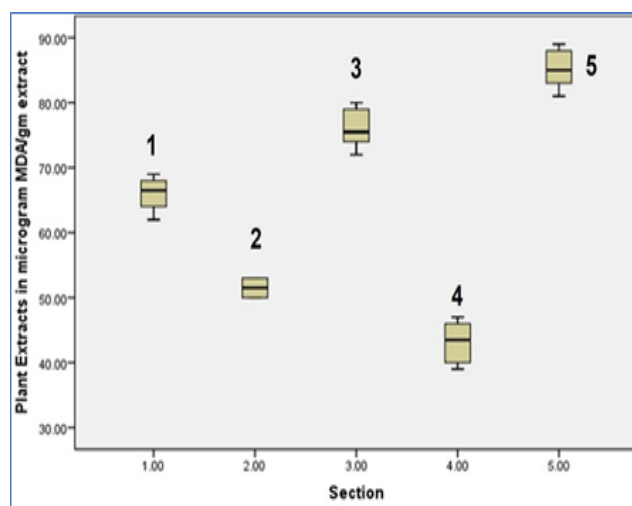
concentration of the experimented compounds that illustrated no perceptible sprouting of bacteria and also no turbidity after a subsequent period of one day of vigorous incubation was envisaged as MIC. Eventually; triplicate repetitions of the analysis were completed for each particular strain (21).

### Statistical Analysis

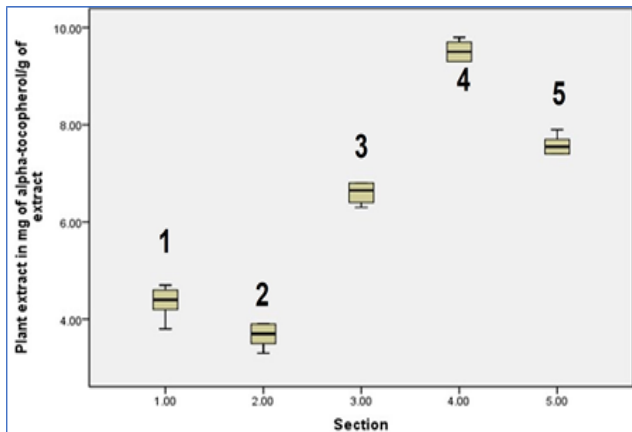
The non-parametric tests (Mann-Whitney U tests and graphs) in SPSS were used.

### Results and discussion

The MDA for *T. coccinea* was  $66 \pm 2.60 \mu\text{g MDA/g}$  extract. For *A. polyacantha*, MDA content was  $51.5 \pm 1.38 \mu\text{g MDA/g}$  extract. It was  $76 \pm 3.03$  for *Polygonum micrpcephallum*. Also, the MDA for *A. spectabilis* showed  $43.15 \pm 3.19 \mu\text{g MDA/g}$  extract. Lastly MDA content for *Clerodendrum colebrookianum* was  $85.17 \pm 3.06 \mu\text{g MDA/g}$  extract. In this parameter; the highest value was found in the extract of *C. colebrookianum* ( $85.17 \pm 3.06 \mu\text{g MDA/g}$  extract) and the lowest value was recorded in the *Abies spectabilis* ( $43.15 \pm 3.19 \mu\text{g MDA/g}$  extract)(Fig. 1 and 2). The TAC for *T. coccinea* was  $4.35 \pm 0.37$ . For *A. polyacantha*; it was  $3.67 \pm 0.24$ . In the case of *P. micrpcephallum*; it was  $6.6 \pm 0.21$ . It was  $10.02 \pm 1.04$  for *A. spectabilis*. Finally; it was  $7.6 \pm 0.19$  for *C. colebrookianum*.

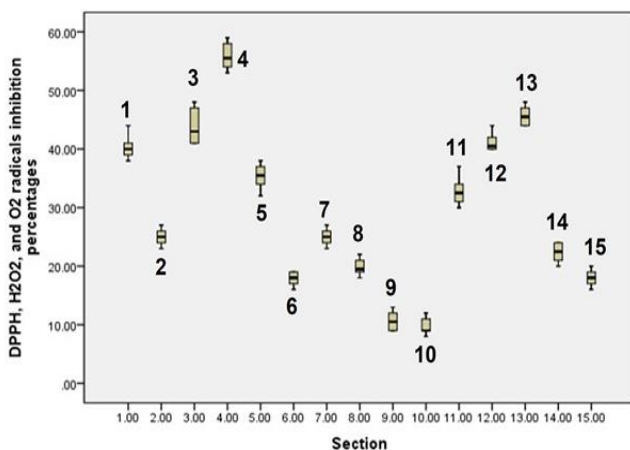


**Figure 1.** Quantity of Malondialdehyde (MDA) in the essential oils of *T. coccinea* (1); *A. polyacantha* (2); *P. micrpcephallum* (3); *A. spectabilis* (4) and *C. colebrookianum* (5).



**Figure 2.** Quantity of TAC in the essential oils of *T. coccinea* (1); *A. polyacantha* (2); *P. micrpephallum* (3); *A. spectabilis*(4) and *C. colebrookianum* (5).

Measurement of DPPH; H<sub>2</sub>O<sub>2</sub>; and O<sub>2</sub>-radicals inhibition percentages were done to analyse the antiradical action of the extracts (Figure 3). Most DPPH scavenging action was unearthed in the extract of *Abies spectabilis* with 55.83 ± 2.31%. Figure 3 depicts that the extract of *A. polyacantha* had excessive H<sub>2</sub>O<sub>2</sub> (25 ± 1.42 %) and *P. micrpephallum* had the maximum O<sub>2</sub>- (45.67 ± 1.63%) radical inhibition percentages.



**Figure 3.** DPPH;H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> radical inhibition percentage of the tested essential oils. Data are means of four replicates (Mean±SE; n=3). From section (1-5): DPPH radical inhibition percentage of *T. coccinea* (1); *A. polyacantha* (2); *P. micrpephallum* (3); *A. spectabilis* (4) and *C. colebrookianum* (5) essential oils. From section (6-10): H<sub>2</sub>O<sub>2</sub> radical inhibition percentage of *T. coccinea* (1); *A. polyacantha* (2); *P. micrpephallum* (3); *A. spectabilis* (4) and *C. colebrookianum* (5) essential oils. From section (11-15): O<sub>2</sub> radical inhibition percentage of *T. coccinea* (1); *A. polyacantha* (2); *P. micrpephallum* (3); *A. spectabilis* (4) and *C. colebrookianum* (5) essential oils.

### Screening of the antibacterial activity by micro broth dilution assay

The antibacterial activity of EEO against the six analysed pathogenic strains showed that *T. coccinea* essential oil had values of MIC ranging from 0.20 ± 0.06 to 5.4 ± 0.20; The range shown by *A. polyacantha* in MIC values was 3.86 ± 0.25 to 6.20 ± 0.16. The range shown by *P. micrpephallum* with MIC had values of MIC ranging from 5.70±0.70 to 8.46 ± 0.20; *A. spectabilis* had values of MIC ranging from 0.25 ± 0.18 to 5.8 ± 0.75. The range shown by *C. colebrookianum* had values of MIC ranging from 3.85 ± 0.15 to 6.45±0.50.

The bactericidal activity demonstrated that *T. coccinea* had MBC values ranging from 0.21 ± 0.06 to 9.70 ± 0.25; *A. polyacantha* had MBC values ranging from 5.44 ± 0.40 to 11.05 ± 0.76; *P. micrpephallum* had MBC values ranging from 10.70 ± 0.55 to 16.30 ± 0.54; *A. spectabilis* had MBC values ranging from 0.25 ± 0.18 to 8.60 ± 0.45; *C. colebrookianum* had MBC values ranging from 7.55 ± 2.2 to 11.54±0.55.

The antibacterial actions determined by paper disc-diffusion technique of the EEO extracted from the essential oils of the five tested plant species exhibits their antibacterial action versus the six tested strains of pathogenic bacteria; i.e.; *S. pyogenes*; *N. gonorrhoeae*; *S. aureus*; *E. coli*; *K. pneumoniae*; and *P. aeruginosa*. But it was seen that; the EEO extracted from *T. coccinea* depicted their antibacterial activity versus all tested pathogenic bacterial strains; with the highest against *S. pyogenes* (22.50±1.60); *A. polyacantha* demonstrated its antibacterial activity against only three bacterial strains: *N. gonorrhoeae*; *P. aeruginosa* and *S. pyogenes*. The essential oil from *P. micrpephallum* also demonstrated good antibacterial activity versus almost all the bacterial strains; with best being against *P. aeruginosa* (22.30±3.40). *Abies spectabilis* essential oil demonstrated good antibacterial activity against all bacterial strains with the highest against *N. gonorrhoeae* (28.60±2.44). It has been seen that antibacterial activity done by the method of disc-diffusion of the EEO extracted from *T. coccinea* was potent versus *S. pyogenes* (22.50±1.60); *N. gonorrhoeae* (18.60±2.48); and *K. pneumonia* (18.60±1.70). Nevertheless; versus *P. aeruginosa* (22.30±3.40); *N. gonorrhoeae* (19.0±2.20) and *S. pyogenes*(19.30±1.64);EEO extracted from *P. micrpephallum* was most potent. Similarly; it was

seen that EEO extracted from *A. spectabilis* was potent against *N. gonorrhoeae* ( $28.60 \pm 2.44$ ) and *K. pneumoniae* ( $20.60 \pm 1.40$ ); EEO extracted from *C. colebrookianum* was most potent against *Neisseria gonorrhoeae* ( $26.80 \pm 1.45$ ) (Table 1 [supplementary file](#)). When the micro broth dilution technique antibacterial activity was tested; the EEO extracted from *T. coccinea* was most potent against *E. coli* ( $4.70 \pm 0.40$ ). Even so; versus *A. baumannii* ( $5.20 \pm 0.50$ ) and *K. pneumoniae* ( $4.93 \pm 0.50$ ); the EEO extracted from *A. polyacantha* was most potent (Table 1). Also; it was seen that the bactericidal action of the EEO extracted from *P. micrcephallum* was most potent against *S. pyogenes* ( $5.70 \pm 0.70$ ); *N. gonorrhoeae* ( $4.44 \pm 0.40$ ); and *E. coli* ( $4.90 \pm 0.76$ ). Again; it was determined that the bactericidal action of the EEO extracted *A. Spectabilis* was most potent versus *A. baumannii* ( $5.76 \pm 0.55$ ) and *S. pyogenes* ( $4.8 \pm 0.40$ ). Nevertheless; versus *E. coli* ( $5.6 \pm 0.36$ ); the bactericidal action of the EEO extracted from *C. colebrookianum* was most potent (Table 1 and 2).

One of the main causes of drug resistance is the large-scale utilization of synthetic drugs to prevent and treat various diseases. Drug resistance to various antibiotics is frequently encountered during antimicrobial therapy. Currently; many medical specialists are looking for substitute treatment modalities for restoring the health of the patients. So; prominence for the increased development of customary herbal medicine from plant sources is on the rise.

Plant sources are proven to be sources of compounds propitious for the health of humans. Regarding antioxidants; this is correlated with a very contemporary issue of averting tumorous situations (neoplasia) (22,23).

One of the most useful results is the positivity of DPPH free radical scavenging activity portrayed by all the plant extracts. DPPH is a crystalline powder that is stable and has a free radical having an unpaired (valence) electron and can rummage and this ability is contemplated as a prime antioxidant property (24). Hydrogen peroxide usually becomes the cause of hydroxyl radicals in the interior of the cell; which is toxic. The rummaging action of  $H_2O_2$  may be ascribed for the most part to the phenolic compounds which can bestow electrons to  $H_2O_2$ ; ultimately converting it to water (25). The xanthine oxidase system is responsible

for the generation of the superoxide anion. The superoxide radical is the precursor of the more harmful ROS such as the hydroxyl radical ( $HO\bullet$ ); singlet oxygen; peroxy nitrite ( $ONOO^-$ ); which are detrimental to the components of the cell components and they cause damage to the tissue and different body diseases (26).

Thiobarbituric acid is utilized to appraise the secondary by-products of oxidation; which are ketone and aldehyde (27). The present research depicted that the least MDA quantity was discovered in the methanolic extract of the dried sample that depicts that this plant extract has the least peroxidation of lipid. DPPH assay is a favorite technique for the screening of antioxidant action of extracts of plants (28). DPPH is one of the least reactive free radicals; hence its reduction can be achieved mainly by more reactive components (reducing components) for example; phenolic substances (29). Many other researchers evaluating plant extracts disclose a depletion in DPPH. The acquired consequences designated that plant extracts with higher TPC had the strongest free radical scavenging effect.

$H_2O_2$  is the least reactive in watery solutions at normal concentrations; has toxicity to cells at levels of 10-100  $\mu$  and is able to cross membranes swiftly to form cytotoxic compounds. Some free radicals; such as  $O_2^-$  and likewise peroxy radical ( $ROO\cdot$ ); are extraordinarily reactive and are renowned to be a biological compound in reducing molecular oxygen (30, 31). However; the results about the  $H_2O_2$  and  $O_2^-$  radicals (in comparison with all other parameters) were found to be true. Interestingly; the greatest scavenging potential of  $O_2^-$  radicals and  $H_2O_2$  were obtained in the fresh samples of other plant extract done in other studies. Even in other extracts; good oxygen radical absorbance activity has been detected (32).

One of the effective MDA determined was the extract of *Clerodendrum colebrookianum* ( $85.17 \pm 3.06$   $\mu$ g MDA/g extract). It was also seen that most DPPH scavenging action was unearthed in the extract of *Abies spectabilis* with  $55.83 \pm 2.31$  %. Fig. 3 depicts that the extract of *Acacia polyacantha* had excessive  $H_2O_2$  ( $25 \pm 1.42$  %) and *Polygonum micrcephallum* had the maximum  $O_2^-$  ( $45.67 \pm 1.63$  %) radical inhibition percentages. Antibacterial activity determined by the method of micro broth dilution of the EEO was also seen to have a positive aspect in these plants. One of

the crucial substitutes to overcome the upraised levels of antibiotic resistance is the discovery of novel metabolites from medicinal plants. The discovery of novel antimicrobial compounds from varied medicinal plants is a replacement to overwhelm the exalted amounts of resistance presented by many human pathogens. The novel antibiotics and correlated chemotherapeutic agents' research are elevating and enriching the chemistry of medicinal plants (33, 34). Medicinal plants are a dominant origin of antioxidants (35). The discovery of different natural antioxidants from plant sources heightens the antioxidant amplitude of the blood and leads to the depletion of the perils from certain infections (36, 37). Different enzymes inhibit the formation of free radicals; some of which are directly involved in the degradation of ROS (primary enzymes); and "secondary enzymes" play an indirect role in supporting other endogenous antioxidants. This is true; for example; of the glucose-6-phosphate dehydrogenase that stimulates NADPH; essential for the initial action of the enzyme. Therefore; the basic molecules present in the different studied plants act in different combinations to aid in the antioxidant activity. Studies in controlled conditions agree that EOs affect the immune system by inducing arachidonic metabolism or cytokine production and/or mutation-mediated genetic mutation by regulating the NF- $\kappa$ B inflammation method and MAPK signaling pathway. Macrophage phagocytosis is also considered possible alternatives to the action of EEOs. Well-designed in vitro studies and in vivo studies; which include a generalization of volume; taking into account environmental factors such as diet; type; and age; will allow for a better understanding of the biological functions of EEOs under several environmental conditions. In addition; the functions of cell activity have not been primarily investigated depth in this field and can change depending on the specific EEO used and the type and the severity of the disease. So; by looking at the quantity of EOs material as well and the scope of the possibilities; there is still a great deal of uncertainty about their true nature effects on health. Further phytochemical; histopathological; and

biochemical investigations are required to fully find out the active principle (s) contained in these plants and discover and learn the mechanism of action(s) to uphold the medicinal value of this plant part.

## Conclusion

The physicochemical constituents; pharmacological and medicinal uses were extensively reviewed in this study. The results that we got from the study look very promising and further studies should be continued in this aspect. With the world population looking for alternative sources; the more medicinal plants we unearth and more medicinal values that we can give to the world will benefit the population of the world. Supplementation of artificial medicines and restoring people to health when emerging diseases create havoc for humanity will benefit. Identifying the most effective combination of specific and specific functional mechanisms is complex due to a large number of molecules with different cellular components; antioxidant and antimicrobial and anti-helminthic functions. Detailed knowledge of the mechanism of action and the effects of individual chemicals may allow for the formation of various chemicals in medicines for the optimal performance and function of EEOs in food.

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**Table 2.** Susceptibility profile of the tested microorganisms against standard antibiotics. *S. pyogenes* (A), *N. gonorrhoeae* (B), *S. aureus* (C), *E. coli* (D), *K. pneumonia* (E) *P. aeruginosa* (F)

Tested organisms	Standard antibiotics; ZOI in mm (mean ± SD)					
	Amoxicillin	Metronidazole	Rifampicin	Clarithromycin	Oxacillin	Clindamycin
A	16.80±1.48	11.80±1.44	22.0±2.5	16.50±1.45	16.40±1.501	11.70±1.50
B	13.65±2.10	9.30±1.22	13.50±1.55	19.50±1.30	12.70±1.25	14.80±2.20
C	13.40±1.60	11.80±2.40	19.50±2.10	18.10±1.25	18.30±1.20	17.60±0.85
D	8.0±1.60	18.40±2.25	17.20±1.60	14.80±1.55	17.50±1.30	18.30±1.55
E	8.30±1.60	18.00±1.36	19.0±1.25	17.40±1.70	16.0±1.25	14.50±1.60
F	13.60±1.50	8.80±1.25	17.20±1.15	16.80±1.35	18.30±1.30	16.70±1.25

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## Supplementary file

**Table 1.** Antibacterial activity of different plant extracted oils against pathogenic bacterial strains.

Microorganisms	Essential oil tested								
	<i>T. coccinea</i>			<i>A. polyacantha</i>			<i>P. micrcephallum</i>		
	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC
<i>N. gonorrhoeae</i>	18.60±2.48	2.24±0.25	3.86±0.25	12.80±1.27	3.96±0.25	9.84±0.60	19.0±2.20	4.44±0.40	14.75±0.25
<i>E. coli</i>	16.44±2.46	4.70±0.40	2.94±0.30	-	4.20±0.16	8.75±0.55	14.70±1.56	4.90±0.76	16.30±0.54
<i>P. aeruginosa</i>	16.40±1.77	0.20±0.06	0.21±0.06	09.90±1.15	3.86±0.25	10.45±2.42	22.30±3.40	3.46±0.20	14.0±0.75
<i>S. pyogenes</i>	22.50±1.60	2.4±0.20	9.40±0.55	11.30±1.14	3.90±0.40	11.05±0.76	19.30±1.64	5.70±0.70	10.70±0.55
<i>K. pneumoniae</i>	18.60±1.70	2.9±0.20	9.70±0.25	-	4.93±0.50	8.04±0.60	7.0±1.25	4.30±0.60	12.45±0.20
<i>A. baumannii</i>	15.40±1.38	2.25±0.20	9.10±0.26	-	5.20±0.50	5.44±0.40	9.20±1.13	4.44±0.40	11.55±0.52
Microorganisms	<i>A. spectabilis</i>			<i>C. colebrookianum</i>					
	ZOI	MIC	MBC	ZOI	MIC	MBC			
<i>N. gonorrhoeae</i>	18.60±2.44	3.24±0.25	5.26±0.25	26.80±1.45	4.36±0.55	9.66±0.70			
<i>E. coli</i>	13.10±2.44	2.40±0.40	4.26±0.30	-	5.6±0.36	8.95±0.25			
<i>P. aeruginosa</i>	17.20±1.55	0.25±0.18	0.21±0.30	17.80±2.10	3.85±0.15	7.55±2.2			
<i>S. pyogenes</i>	15.40±1.60	4.8±0.40	6.30±0.55	14.40±2.15	4.10±0.20	11.05±0.25			
<i>K. pneumoniae</i>	20.60±1.40	2.8±0.75	8.60±0.45	-	4.90±0.60	09.25±0.80			
<i>A. baumannii</i>	15.80±1.55	5.76±0.55	7.80±0.46	-	2.45±0.50	11.54±0.55			

\*: ZOI in mm (mean±SD); MIC and MBC are expressed in mg/ml.