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Research article

ANTIBACTERIAL ACTIVITIES AND GC-MS ANALYSIS OF PHYTOCOMPONENTS OF *EHRETIA ABYSSINICA* R.BR. EX FRESEN

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ABSTRACT: The *Ehretiaabyssinica*, a small tree, belong to Boraginaceae family is traditionally known for its medicinal properties. The present study was therefore carried out to investigate the antibacterial activities and to unveil the Phytochemicals of the bioactive components in the leaves extract of this plant species. The antimicrobial activities of leaves extract was investigated against 7 medically important bacterial strains, namely *Bacillus subtilius, MRSA, Micrococcus ,Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aurues* and *Klebsella pneumoniae*. The antibacterial activity was determined using agar well diffusion method. The most susceptible bacteria to this extract were *Pseudomonas aeruginosa*, followed by *Staphylococcus aurues*, while the most resistant bacteria were *Micrococcus*. GC-MS analysis revealed that the ethanol leave extract of *Ehretiaabyssinica* contained mainly Octadecenamide (5.77%); Lucenin 2 (5.46%); Docosane and Nonacosane (3.75%); Cyclopropene (3.50%); Hematoporphyrin (2.68%); Tetratetracontane (2.36%); Dotriacontane (1.57); Acetic acid (1.53); Nmethylglycine (1.49%); Propyne antimicrobial (1.41%). All identified compounds are known to have antimicrobial activity.
Key words: *Ehretiaabyssinica*, antimicrobial activities, phytochemicals, GC-MS analysis.

INTRODUCTION

The use of medicinal plants as a source of medicine has been inherited and is an important component of the health care system. Recently, extracts and bioactive compounds which isolated from medicinal plants are used for antibacterial, antifungal and antiviral therapy (Asres.*et al*., 2001; Bratner and Grein, 1994). The genus *Ehretia* Linn. (Boraginaceae, subfamily Ehretiaceae) contains about 50 species mainly distributed in tropical Asia and Africa (Gurke, 1893). All species of the genus are trees or shrubs. The leaves, barks, roots, branches, fruits, and heartwoods are used as the herbal medicines separately (Wang, 1979). Literature survey revealed wide biological activity of family Boraginaceae. *Ehretia* spp is a small tree, it is generally found in Asia and Australian tropics, the inner bark is used as food, leaves are applied to ulcers and in headache (Joshi, 2000). The quantitative estimation of phytoconstituents and trace elements of the leaves study establishes the nutritional value to contribute as the resources of Fats, Proteins, Carbohydrates, Vitamin-C, E, A, Riboflavin and Thiamine(Torane,2010). The leaves have been examined to know the presence of constituents. In the present study, ethanol extract of the leaves of *Ehretia abyssinica* was analyzed. This work will help to identify the compounds, which may be used in therapeutic value. GC-MS is one of the best techniques to identify the constituents of ethanol extract. It is composed of long chain, branched hydrocarbons, alcohol, fatty acid and esters.

MATERIALS AND METHODS

Collection and preparation of samples

Samples of leaves of *Ehretiaabyssinica* was collected during March, 2013 from Albahah province (19° 45' 16.61" N, +41° 27' 30.95"E) Southwest Saudi Arabia from warm foothills 350 M.A.S.L. Species status of this plant was fervid at Faculty of Sciences Herbarium (Serial No. 1597), King Abdulaziz University, Jeddah. The plant leaves was brought to the laboratory, washed in running tap water to remove debris and dust particles and then rinsed in distilled water for 5 min, then removed and air dried under room temperature until constant weight.

Extract preparation

Ten grams of dried leaves was thoroughly washed in running water prior to cutting into small pieces (1 to 2 mm) by blender. Extraction was done by adding 100 ml ethanol then each was filtered through a filter paper. The extract solutions were evaporated under reduced pressure at 40°C until dryness; subsequently, the extract was diluted by dimethylsulfoxide (DMSO) and stored at 20°C. Until analysis (Boeru and Derevici, 1978).

Bacterial strains

Bacterial cultures were prepared for in vitro antibacterial assay of the six Bacteria strains four Gram positive: *Bacillus subtilis* (ATCC11774); *S.aureus* (ATCC29213); *Micrococcus* and MRSA (ATCC4698) and three Gram negative *Escherichia coli* (ATCC8739); *Klebsiella pneumonia* (ATCC700603) and *Pseudomonas aeruginosa* (ATCC27853). Those strains were provided by Microbiologics® USA. Tested organisms were sub-cultured on nutrient agar (Oxoid laboratories, UK) slopes. These stock cultures stored in the dark at 4°C until used.

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce (1994). DMSO was used as a negative control and streptomycin and ciprofloxacin (10 mg/disc) were used as a positive control for bacterial strains, the plates were done in triplicate. Bacterial cultures were incubated at 37°C for 24 h. Solutions of 10 mg /ml of streptomycin and ciprofloxacin were used as standard for comparison. Antimicrobial activity was determined by measurement zone of inhibition (Agwa *et al.*, 2000).

Gas chromatography-Mass spectrometry analysis

Extraction of the volatile constituents:

200g of fresh leaves of *Ehretia abyssinica* was extracted with ether (Harborne, 1973). The extract was filtered and the solvent was removed under reduced pressure at 30°C after dehydration over anhydrous sodiumsulphate. The yielded quantity was subjected to GC/MS spectrometer.

Investigation of the volatile constituents

Volatiles were analyzed by GC/MS .The constituents were identified by comparing their retention times and mass fragmentation patterns with those of the database libraries [Wiley (Wiley Int.USA] and the published data (Adams, 1995). Quantitative determinations were carried out based on computerized peak area measurements.

Statistical analysis

For each treatment, three replicates and three determinations were conducted. Means of variable, standard error was calculated using SPSS to authenticate the significant differences between both the pathogenic microorganisms and extract.

RESULTS AND DISCUSSION

Antimicrobial activities

The antimicrobial activities of leaves extracts of *Ehretiaabyssinica*, obtained with the ethanol extract against the tested bacterial strains (Table-1). Ethanol extracts in this study might have had higher solubility for more phytoconstituents, resulting, the highest antibacterial activity. These studies confirm the results of Rajib *et al*. (2009) and Jigna *et al*. (2005).Ethanol extract showed best activity against *P. aeruginosa* >*S.aureus* > *K. pneumoniae* > *E.coli* > *MRAS*>*B.subtilius*>*Micrococcus*. Among the Gram-positive bacteria, these results of Iqbal and Arina (2001)stated that the sensitivity of test strains was *S.aureus* >*Staphylococcus dysenteriae*>*Staphylococcus paratyphi*> *B. subtilius* Similar to our results, *B. subtilius* was least sensitive compared to other test bacteria, which may be due to their ability to form highly resistant resting stagecalled endospores. The ethanol extracts of leaves of *Ehretiaabyssinica* were also tested on three Gram-negative bacteria; showed a strong activity against *P.aeruginosa* followed by *K.pneumoniae* and *E.coli.* The plant extracts inorganic solvent provided more consistent antimicrobial activity. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay (Jigna *et al*., 2005). Similar results have been reported in previous studies (Ahmed *et al.*, 1998).

The difference in potency of plant extracts may be due to different sensitivity of the tested strains and methods of extraction (Nimri *et al*., 1999). Antimicrobial activities of standard antibiotics showed an inhibitory effect against all the tested bacteria. The results also showed that the ciproflaxocin is more effective than streptomycin. The present results go in line with Shital (2010). From Table (1)seven drug-resistant strains of bacteria were found to be sensitive to the tested plant extracts; this has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms (Iqbal and Arina, 2001).

Previously, antimicrobial activities of 50 Taiwanese folk medicinal plants were examined and screened for 10 strains of *Helicobacter pylori*, *Ehretia abyssinica* demonstrated moderate anti- *H.pylori* activities against two strains, lower anti- *H.pylori* effects against sex strains and no activity against two strains by Yuan and Tung (2006).The present results go in line with Gumgumjee *et al.* (2012) stated that *Tamarindusindica* leaves ethanol extract has produced strong antibacterial effects against *S.aureus*> MRSA> *B.subtilius* respectively. *S.aureus* showed also similar results when treated with *Casuarinaequisetifolia* leaves ethanol extract (Gumgumjee and Hajar, 2012). The ethanol extracts of different parts of *Conocarpus erectus* were also tested on Gram – ve bacteria; *E.coli, K. pneumoniae* and *P.aeruginosa*. The results showed a strong effect against *P. aeruginosa* followed by *E.coli* and *K. pneumoniae* at 200 µl /dish by all tested parts of *Conocarpus erectus* bark extracts (Hajar and Gumgumjee, 2013). The present results agreed to some extent with El-Sayed *et al*. (2012). The susceptibility of bacteria strains to plant species extracts may draw attention to plants potentials as a natural antibiotic producers, these can be used against the susceptible bacteria strains (Khosravi & Behzadi, 2006). Due to the nature of the bacteria species response, differences between them are expected (Moyo *et al*., 2012). Results obtained from present study provide more evidence on the antimicrobial efficacy of *Ehretiaabyssinica*. The mechanisms of actions of these compounds have been proven to be via cell membranes perturbations (Esimone *et al*., 2006).Thus, our antimicrobial screening results also justifies the traditional uses of these plants in various ailments including infectious disease. Further, the active phytocompounds of these plants against multidrug-resistant bacteria to be characterized and the efficacy of non-toxic extracts preparations has to be evaluated in *vivo*. In additions Sivasankari *et al*., (2013) showed that the methanol extract of *Ehretiaabyssinica* was found to have high antioxidant potential. This antioxidant potential along with good phenolic content tends to pave this wild plant fruit suggested for domesticate and health supplement which will definitely fragile the disease prone. The *Ehretiaabyssinica* extracts can be subjected to isolation of the therapeutic, antimicrobials and undergo further pharmacological evaluation.

Table-1: The antibacterial activity of ethanolic extract of *Ehretia abyssinica* concentration 100mg/ml compared to Antibiotics against different pathogenic bacteriai.

Types of Bacteria	Pathogenic Bacteria	Leaves extract	Streptomycin (10ug/disc)	Ciprofloxacin (10ug/disc)
Gram positive bacteria	***Bacillus subtilius***ATCC11774	15.33±0.33	25.00 ± 0.00	34.00 ± 0.00
	MRSA(ATCC977)	19.33±0.33	20.00 ± 0.00	36.00 ± 0.00
	Staphylococcus aureusATCC29213	24.76±0.33	19.00 ± 0.00	38.00 ± 0.00
	Micrococcus ATCC4698	14.33±0.33	27.00 ± 0.00	46.00 ± 0.00
Gram negative bacteria	***Echerichia coli ATCC8739***	20.67±0.33	23.00 ± 0.00	44.00 ± 0.00
	KlebsiellapneumoniaeATCC700603	23.67±0.33	25.00 ± 0.00	42.00 ± 0.00
	PseududomonasaeruginosaATCC27853	26.33±0.33	22.00 ± 0.00	42.00 ± 0.00
Mean of type of the extract	20.63		23.00	40.29

GC-MS analysis

GC-MS chromatogram of the ethanolic extract of *Ehretiaabyssinica* leaves was showed on comparison of the mass spectra of the constituents with the NIST library, eleven peaks were obtained; all the phytocomponents were characterized and identified (Table 2). The retention times (RT) are in minutes.

Table 2: Phytocomponents identified in the ethanolic leaf extract of *Ehretia abyssinica* by GC-MS.

No	Rt	Name of the compound	Molecular Formula	Molecular Weight	Area (%)
1	45.31	9-Octadecenamide	C18H35NO	281	5.77%
2	53.80	Lucenin 2	C27H30O16	610	5.46%
3	53.20	Docosane	C22H46	310	3.75%
4	53. 54	Nonacosane	C29H60	408	3.75%
5	12.36	Cyclopropene	C3H4	40	3.50%
6	5.13	Hematoporphyrin	C34H38N4O6	598	2.68%
7	48. 90	Tetratetracontane	C44H90	618	2.36%
8	54. 50	Dotriacontane	C32H66	450	1.57%
9	5.59	Aceticacid	C31H46O4	482	1.53%
10	10. 30	Nmethylglycine	C3H7NO2	89	1.49%
11	49.18	Flavone	C27H30O15	594	2.02%

Table 3: Activity of chemical constituents identified in the ethanolic extract of *Ehretia abyssinica* by GC- MS analysis

Rt	Name of compound	Activity
45.31	Octadecenamide	Antibacterial activity (Waage&Hedin,1985).
53.80	Lucenin2	Antibacterial activity (Waage&Hedin,1985).
53.20	Docosane	Antibacterial activity (Waage&Hedin,1985).
53. 54	Nonacosane	Antibacterial activity (Waage&Hedin,1985).
12.36	Cyclopropene	Antibacterial activity (Waage&Hedin,1985).
5.13	Hematoporphyrin	Antimicrobial activities against Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* (Waage & Hedin, 1985).
53. 97	Tetratetracontane	Promoted an effective action in bacterial reduction with the application of laser energy (Sun *et al*., 2013).
54. 50	Dotriacontane	Antimicrobial agent, hypercholesterolemic (Ngassoum *et al*.,2000)
5.59	Acetic acid	Antimicrobial and antioxidant activities(Ngassoum *et al*.,2000)
10. 30	Nmethylglycine	Disinfection of wounds and antiseptic agent in the treatment and prophylaxis of the plague (Russel *et al*., 2009).
49.18	Flavone	Antimicrobial activities (Yanug*et al*., 2007).

The major constituents was found to Octadecenamide (5.77 %), Lucenin 2 (5.46 %), Docosane – Nonacosane (3.75 %) and cyclopropene (3.50)whose retention time are found at 45.31,53.80,53.20,53. 54and 12.36 respectively Fig (1).Flavonoids are an important part of volatiles from plants. The Flavonoid tested Lucenin 2 are more active on Gram-negative bacteria contrary to literature reports, indicat- ing that Gram-positivebacteriaare selectively inhibited by Flavonoids and isoflavonoid derived from plants (Waage & Hedin, 1985).Acetic acid has been commonly used in medicine for more than 6000 years for the disinfection of wounds and especially as an antiseptic agent in the treatment and prophylaxis of the plague. The microbiological spectrum of acetic acid is wide, antiseptic solutions, it showed similar - in some bacteria, even better- bactericidal properties. An evaluation of the clinical value of topical application of acetic acid is currently underway. It can be concluded that acetic acid has excellent bactericidal effect and, therefore, seems to be suitable as local antiseptic agent (Russel *et al*., 2009) .Hematoporphyrinmonomethyl ether (HMME) as a novel and affordable photosensitizer has been used in treating various clinical diseases for years. Its bactericidal effects on the pathogenic microbes in supragingival plaque which can lead to many oral infectious diseases such as caries, gingivitis (Sun *et al*., 2013).

Cyclopropene well know as antibacterial, antifungal, antiviral, antibiotics, antitumer, insecticidal and herbicidal activities (Salaun and Baird, 1995). Nonacosane is a straight-chain hydrocarbon it plays a role in the chemical communication of several insects, including the female *Anopheles stephens*i mosquito Brei *et al*., (2004). Nonacosane occurs naturally and has been identified within several essential oils. It can also be prepared synthetically Bentley *et al*.; (1995). Flavone shows an antimicrobial effect on *S. cerevisiae*. Flavonoids including flavones have antioxidative effects, (Yanug *et al*., 2007). Flavonoids possessing a diversite range in pharmaceutical properties compounds with antifungal, antibacterial and antivirial activity (Tim and Anderow, 2005). Tetratetracontane provide the fundamental pharmacodynamic data for the toosendanin application as an antimicrobial agent and hypercholesterolemic. components, n- Dotriacontane has been previously reported from *Laggera aurita*, while other volatiles like sabinene, p-cymene, gamma-eudesmol were previously reported from the Cameroonian species of the plant (Ngassoum *et al.*, 2000). Dotriacontane has been previously reported from *Laggeraaurita*, its shows strong an antimicrobial effect (Ngassoum *et al*., 2000).

The present study has been found useful in the identification of several constituents present in the ethanolic extract of the leaves of *Ehretiaabyssinica*. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. It could be concluded that *Ehretia abyssinica* plant is of phyto pharmaceutical importance. Studies with these compounds may yield nature friendly strong anti-bacterial agents of agricultural importance.

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