Bio Bulletin 2(2): 08-12(2016)
(Published by Research Trend, Website: www.biobulletin.com)

ISSN NO. (Print): 2454-7913
ISSN NO. (Online): 2454-7921

Antibacterial Activity and Phytochemical Constituents of *Cinnamomum verum* and *Matricaria chamomilla* from Sudan

Rania Mohamed Abdalla* and Abdelgadir Elfadil Abdelgadir**
**Department of Biology and Biotechnology,*
Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan.
***Department of Environmental science,*
Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan.

(Corresponding author: Rania Mohamed Abdalla, rania.sci79@yahoo.com)
(Published by Research Trend, Website: www.biobulletin.com)
(Received 19 May 2016; Accepted 22 July 2016)

ABSTRACT: The present study describes the phytochemical profile and antibacterial activity of the bark of *Cinnamomum verum* and flowers of *Matricaria chamomilla* profusely prescribed in traditional medicine in Sudan. Our phytochemical investigations revealed many principles of bioactive properties in animal and human, such as sterols, triterpens, flavonoids, tannins and alkaloids for *Cinnamomum verum*, sterols, triterpens, flavonoids, saponins, tannins and alkaloids for *Matricaria chamomilla*. The antibacterial activity was examined using agar well-diffusion method, using different extracts; Petroleum ether, ethyl acetate, methanol and ethanol against two gram positives (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 8236) and two gram negatives (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), the results showed that most extracts have significant antibacterial activity. The current study supports the employment of these plants in the Sudanese folk medicine and recommends further microbiological and pharmacological studies as a promising sources for new antibacterial agents.

Key Words: Antibacterial, *Cinnamomum verum*, *Matricaria chamomilla*, phytochemical analysis

INTRODUCTION

Plants have provided human beings with all his needs in terms of shelter, clothing, food, flavors and fragrances and not the least, medicines. These plant-derived drugs have given rise to some important drugs still in use today. The search for new molecules, nowadays, has taken a slightly different route where the science of ethno-pharmacology is being used as a guide to lead the biomedical researchers towards different sources and classes of compounds (Gurib-Fakim, 2006). The earliest recorded knowledge obtained from countless prehistoric references from ancient Egypt, Assyria, China and India is showing numerous prescriptions from medicinal plants to be used against different ailments (Heinrich *et al.,* 2004). In Sudan, traditional medical practices play an important role, about 90% of the population, particularly those who are living in frontiers and rural areas depend mainly on the traditional herbal medicine for the treatment of various types of ailments and disorders (Koko *et al*., 2000). Nowadays, due to the recent failure of antibiotics against resistant bacteria beside the dramatic spread of these multidrug resistant pathogens, urge the health organizations and pharmaceutical industries all over the world to search for alternative drugs, from medicinal plants in particular (Abdallah, 2011).

Chamomile (*Matricaria chamomilla*) which belonging to family Asteraceae, is one of the most ancient medicinal herbs known to mankind (Fatouma *et al.,* 2011). Chamomile flowers are taken as herbal tea, the flowers contains 1-2% volatile oils including alpha-bisabolol, alpha-bisabolol oxides, and matricin, and also rich in flavonoids (Lemberkovics *et al*., 1998). Chamomile has also numerous applications in traditional medicine; It is used as anti-cold (Saller *et al*., 1990), for gastrointestinal and digestive disorders (Kroll and Cordes, 2006), against Eczema (Nissen *et al*., 1988), anti-estrogenic effect (Kassi *et al*., 2004), anti-diabetic (Kato *et al*., 2008), for wound healing (Martins *et al*., 2009) and as an anti-cancer (Way *et al*., 2004).

Cinnamon (*Cinnamomum verum*) which belonging to family Lauraceae is a famous plant worldwide. *Cinnamomum verum* is rich in essential oils, resinous compounds, Cinnamic acid, Cinnamaldehyde and Cinnamate, it has been added to different types of food to impart flavor as well as to improve storage stability, since ancient times (Jakhetia *et al*., 2010). Cinnamon has many applications in traditional medicine, such as antimicrobial (Matan *et al*., 2006), anti-oxidant (Madhavi and Salunkhe 1995), anti-diabetic (Subash *et al*., 2007) and anti-inflammatory (Tung *et al*., 2008). The aim of this study is to evaluate the potential antibacterial activity of both plants, which are used in the traditional Sudanese medicine.

MATERIALS AND METHODS

A. Collection of plant materials

The bark of *Cinnamomum verum* and the flowers of *Matricaria chamomilla* were purchased from the herbal medicine shop (Attar) from Khartoum North town. Plants were then identified and authenticated by the herbalists at Medicinal and Aromatic Plants Research Institute, The National Center for research, Kartoum, Sudan.

B. Preparation of the extracts

Extraction was carried out according to the method described by Sukhdev *et al*. (2008). Briefly, 100 g of each of *Cinnamomum verum* and *Matricaria chamomilla* was successively extracted with petroleum ether, ethyl acetate and methanol using soxhelt extractor apparatus. Then, extracts were evaporated under reduced pressure using rotary evaporator apparatus and allowed to dry in the incubator till complete dryness.

Then, the dried extracts were reconstituted in 10% DMSO (Di-methylesulphoxide) to get a final concentration 100 mg/ml from each extract. The 10% DMSO is not lethal to bacteria (Abdallah *et al*., 2009).

C. Phytochemical analysis

Qualitative phytochemical screening of some bio-active principles were carried out using the methods reported by Martinez and Valencia (2003), Sofowora (1993), Harborne (1984) and Wall *et al* (1952).

D. Preparation of bacterial cultures

One ml aliquots of 24 h broth culture of testing organisms were aseptically added to nutrient agar slopes and incubated (griffin and George Ltd, England) at 37°C for 24 h. The tested bacteria were two gram positives (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 8236) and two gram negatives (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). The bacterial growth was harvested and washed off by the addition of sterile normal saline. The harvested bacteria were suspended in a suitable volume of normal saline to prepare a suspension containing about 108 -109 colony forming units per ml (CFU\ml). The suspension was stored in the refrigerator at 4 °C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra 1938).

E. Antibacterial activity

The cup-plate agar diffusion method (Kavanagh, 1972) was used with some minor modifications to evaluate the antibacterial activity of the extracts. 1 ml of the standardized bacterial stock suspension (108 -109 C.F.U/ ml) were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4), and agar discs were removed. Alternate cup was filled with 0.1 ml sample of each extract and allowed to diffuse at room temperature for two hours. The plates were then incubated for 18 hours at 37ºC. After incubation, the diameters of the growth inhibition zones were measured and averaged and the mean values of two replicates were recorded. Ampicillin and Gentamicin discs (10 µg/disc) were used as positive controls, while discs saturated with 10% DMSO were used as negative controls.

RESULTS AND DISCUSSION

As shown from Table 1, the phytochemical investigation revealed many compounds of bio-active properties. *Matricaria chamomilla* revealed presence of sterols, triterpens, flavonoids, saponins, tannins and alkaloids. This is in harmony -partially- with Vinha *et al*. (2012) who detected steroids, terpenoids, flavonoids and tannins, but no alkaloids.

Table 1: The result of phytochemical screening.

Phytochemical	*Matricaria chamomilla*	*Cinnamomum verum*
Sterols	+	++
Triterpenes	+++	+
Flavonoids	++	++
Saponins	+	-
Cumarins	_	-
Tannins	++	+
Anthraquenones	-	-
Alkaloids	+	+

+ Traces, ++ Moderate, + ++ High, - Negative

Differences in geographical areas may lead to variation in the chemical contents of the plant. On the other hand, *Cinnamomum verum* showed the presence of sterols, triterpens, flavonoids, tannins and alkaloids. This is in agreement with Harsha *et al* (2013) where most of these phytochemicals detected. Plants rich in phytochemical compounds often characterized by medicinal properties and such plants are worthy of biomedical investigations, which may lead to new drugs (Vinha *et al*., 2012). It was found that the antimicrobial activities of medicinal plants are attributed to some phytochemical compounds such as alkaloids, flavonoids, saponins and tannins (Abalaka *et al*., 2012). The results of agar -well diffusion test are presented in Table 2.

Table 2: Antibacterial activity of methanolic, ethanolic, petroleum ether and ethyl acetate extracts of *Cinnamomum verum.*

Test	Concentration	Standard tested organisms* M.D.I.Z (mm)**			
		B.s	**S.a**	**E.c**	**P.s**
Ethanol extract	100mg/ml	22±2.0	25± 3.5	25±2.5	-
Methanol extract	100mg/ml	-	-	-	-
Petroleum ether extract	100mg/ml	26±5.0	25±4.5	23±3.0	22±2.0
Ethyl acetate extract	100mg/ml	25±5.5	26±4.0	32±7.0	30±8.5
DMSO	10%	-	-	-	-
Ampicillin	10 µg/disc	13±1.0	18±2.0	-	12±2.0
Gentamicin	10 µg/disc	20±0.0	30±3.0	17±3.0	21±1.5

*Standard organisms tested: B.s= *Bacillus subtilis*, S.a = *Staphylococcus aureus*, E.c = *Escherichia coli*, Ps.a = *Pseudomonas aeruginosa*.

** M.D.I.Z=: Mean diameter of growth inhibition zone in (mm)± Standard deviation. Interpretation of results: MIZD (mm) >18 mm: Sensitive, 14 – 18 mm: Intermediate, < 14 mm : Resistant, (-) : No inhibition zone.

The ethanol, petroleum ether and ethyl acetate extracts of *Cinnamomum verum* revealed different degrees of antibacterial activities against tested bacteria, while the methanol did not show any obvious inhibition zones. On the other side, the ethanol, methanol and petroleum ether of *Matricaria chamomilla* showed different degrees of antibacterial activities against bacteria understudy, whereas, the ethyl acetate did not show any obvious antibacterial activities.

Table 3: Antibacterial activity of methanolic, ethanolic, petroleum ether, ethyl acetate extract of *Matricaria chamomilla.*

Test	Concentration	Standard tested organisms* M.D.I.Z (mm)**			
		B.s	S.a	E.c	P.s
Ethanol extract	100mg/ml	17±2.0	19±2.0	20±5.0	18±3.0
Methanol extract	100mg/ml	17±0.0	-	17±2.0	17±1.0
Petroleum ether extract	100mg/ml	26±7.5	25±5.0	23±4.0	22±3.5
Ethyl acetate extract	100mg/ml	-	-	-	-
DMSO	10%	-	-	-	-
Ampicillin	10 µg/disc	13±1.0	18±2.0	-	12±2.0
Gentamicin	10 µg/disc	20±0.0	30±3.0	17±3.0	21±2.5

*Standard organisms tested: B.s= *Bacillus subtilis* S.a = *Staphylococcus aureus*, E.c = *Escherichia coli*, Ps.a= *Pseudomonas aeruginosa*.
** M.D.I.Z=: Mean diameter of growth inhibition zone in (mm) ± Standard deviation. Interpretation of results: MIZD (mm) >18 mm: Sensitive, 14 – 18 mm: Intermediate, < 14 mm: Resistant, (-) : No inhibition zone.

This may be attributed to the nature of the phytochemical constituents of the tested plants, which showed different components. A quantitative phytochemical analysis and fractionation of the extracts of both plants are required in order to evaluate the antibacterial efficacy more precisely. The negative control, DMSO (10%) did not show any antibacterial effect, while the positive control; Ampicillin and gentamicin (10µg/disc) showed antibacterial activity. However, gentamicin was much effective. Many previous studies from different countries around the world reported some antibacterial activities of these plants against different types of bacteria. Ismail *et al*. (2011) reported that water extract of *Matricaria chamomilla* showed a potent inhibitory effect against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and Escherichia coli. Rakshit and Ramalingam (2011) mentioned that aqueous extract of bark of *Cinnamomum verum* revealed good antibacterial effects against food borne pathogens, which were three gram positive (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis*) and two gram negative (*Escherichia coli, Proteus mirabilis*). Accordingly, the *Matricaria chamomilla* and *Cinnamomum verum* are a good sources for antibacterial agents and the current study gives a scientific evidence of using these plants in the Sudanese traditional medicine as well as its traditional applications worldwide.

CONFLICT OF INTEREST

There are no conflicts of interest regarding the contents of this article.

ACKNOWLEDGEMENTS

The authors would like to thank the lab technicians and staff members at the Medicinal and Aromatic Plants Research Institute, The National Center for research, Khartoum, Sudan. Thanks also to all staff members and technicians at school of biology and biotechnology, college of science and technology, Al-Neelain University, for cooperation and guidance.

REFERENCES

Abalaka, M.E., Daniyan, S.Y., Oyeleke, S.B., Adeyemo, S.O. 2012. The antibacterial evaluationof *Moringa oleifera* leaf extracts on selected bacterial pathogens. *J. Microbiol. Res.* **2**(2): 1-4.

Abdallah, E.M. 2011. Plants: An alternative source for antimicrobials. *J. Applied Pharma. Sci.* **1**(6): 16-20.

Abdallah, E.M., Khalid, A.S., Ibrahim, N. 2009. Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswelliapa pyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA). *Sci. Res. Essay*. **4**(4): 351-356.

Fatouma, M., Abdoul-Latif, N.M., Prosper, E.P., Adwa, A.A., Samatar, O.D., Louis-Clément, O., *et al*. 2011. Antimicrobial and antioxidant activities of essential oil and methanol extract of *Matricaria chamomilla L*. from Djibouti, *J. Med. Plants Res.* **5**(9): 1512-1517.

Gurib-Fakim, A. 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* **27**, 1-93.

Harborne, J.B. 1984. Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd edition, London New York.

Harsha, N., Srideci, V., Lakshmi, M.V.V.C., Rani, K., Vani, N.D.S. 2013. Phytochemical analysis of some selected spices. *Inter. J. Innovative Res. Sci. Eng. Tech.* **2**(11): 6618-6621.

Heinrich, M., Barnes, J., Gibbons, S., Williamson, E.M. 2004. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK.

Ismail, M.C., Ibrahim, K.M., Lateef, N.S. 2011. Effect of Flower Extracts of *Matricaria chamomilla* L. on Some Bacteria Causing Eye Infections. *Diyala J. Pure Sci.* **7**(3): 97-109.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A., Sharma, S. 2010. Cinnamon: a pharmacological review. *J. Advanced Sci. Res.* **1**(2): 19-23.

Kassi, E., Papoutsi, Z., Fokialakis, N., Messari, I., Mitakou, S., Moutsatsou, P. 2004. Greek plant extracts exhibit selective estrogen receptor modulator (SERM)-like properties. J. Agricult.Food Chem. 52: 6956-6961.

Kato, A., Minoshima, Y., Yamamoto, J., Adachi, I., Watson, A.A., Nash, R. J. 2008. Protective effects of dietary chamomile tea on diabetic complications. *J. Agricult. Food Chem*. **56**: 8206-8211.

Kavanagh, F. 1972. Analytical Microbiology. Vol. **2**, Acad. Press, New York, USA.

Koko, W.S., Galal, M., Khalid, H.S. 2000. Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanitesa egyptiaca* compared with albendazole. *J. Ethnopharma*. **71**: 247-52.

Kroll, U.,Cordes, C. 2006. Pharmaceutical prerequisites for a multi-target therapy. *Phytomedicine* 13(5): 12-19.

Lemberkovics, E., Kery, A., Marczal, G., Simandi, B., Szoke, E. 1998. Phytochemical evaluation of essential oils, medicinal plants and their preparations. *Acta Pharma Hung.* **68**(3): 109-141.

Madhavi, D.L., Salunkhe, D.K. 1995. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK. (Eds.), Food Antioxidants. Marcel Dekker Inc., New York, p. 267.

Matan, N., Rimkeeree, H., Mawson, A.J., Chompreeda, P., Haruthaithanasan, V., Parker, M. 2006. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *Int. J. Food Microbiol*. **107**: 180-185.

Martins, M.D., Marques, M.M., Bussadori, S.K., Martins, M.A., Pavesi, V.C., Mesquita-Ferrari, R.A., Fernandes, K.P. 2009. Comparative analysis between *Chamomilla recutita* and corticosteroids on wound healing: An in vitro and in vivo study. *Phytother. Res.* **23**(2): 274-278.

Martinez, A., Valencia, G. 2003. Manual de prácticas de Farmacognosia y Fitoquímica: 1999. 1. Medellin: Universidad de Antioquia; *Marcha fitoquimica*; pp. 59-65.

Miles, A.A., Misra, S.S. 1938. The estimation of the bactericidal power of the blood, *J. Hygiene,* **38**: 732-749.

Nissen, H.P., Biltz, H., Kreysel, H.W. 1988. Profilometry, a method for the assessment of thetherapeutic effectiveness of Kamillosan ointment. Z Hautkr. **63**(3):184-190.

Rakshit, M., Ramalingam, C. 2011. In-vitro antibacterial and antioxidant activity of *Cinnamum vernum* (Cinnamon) aqueous bark extract in reference to its total phenol content as natural preservative to food. *Int. J. Biol. Biotech.* **8**(4): 529-537.

Saller, R., Beschorner, M., Hellenbrecht, D., Bühring, M. 1990. Dose-dependency of symptomatic relief of complaints by chamomile steam inhalation in patients with common cold. *Eur. J. Pharmacol.* **183**: 728-729.

Sofowora, A. 1993. Medicinal Plants and Traditional Medicines in Africa.Chichester John, Willey and Sons, New York, USA.

Subash, B.P., Prabuseenivasan, S., Ignacimuthu, S. 2007. Cinnamaldehyde-a potential antidiabetic agent. *Phytomedicine.* **14**: 15-22.

Sukhadev, S.H., Suman, P.S.K., Gennaro, L., Dev, D.R. 2008. An overview of extraction techniques for medicinal and aromatic plants. In: Sukhdev SH *et al*. (Eds). Extraction technologies for medicinal and aromatic plants. International Center for Science and High Technology. Trieste, Italy.

Tung, Y.T., Chua, M.T., Wang, S.Y., Chang, S.T. 2008. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresour. Technol.* **99**(9): 3908-3913.

Vinha, A.F., Soares, M.O., Castro, A., Santos, A., Oliveira, M.B.P.P., Machado, M. 2012. Phytochemical Characterization and Radical Scavenging Activity of Aqueous Extracts of Medicinal Plants from Portugal. *Eur. J. Med. Plants.* **2**(4): 335-347.

Wall, M.E., Eddy, C.R., McClenna, M.L., Klump, M.E. 1952. Detection and estimation of steroid and sapogenins in plant tissue. *Analytical Chemistry,* **24**: 1337-1342.

Way, T.D., Kao, M.C., Lin, J.K. 2004. Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-over-expressing breast cancer cells via the phosphatidylinositol-3'-kinase/Akt-dependent pathway. *J. Biol. Chem*. **279**: 4479-4489.