International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 56.67 | Impact Factor (2017): 5.156

Phytochemical and Antimicrobial Evaluations of Some Medicinal Plants in Surigao del Sur, Philippines

Gemma A. Gruyal

Surigao del Sur State University-Cantilan Campus, Department of Biological Science, College of Teacher Education
Cantilan, Surigaodel Sur 8317, Philippines-083-212-5132

Abstract: ***Medicinal plants have been widely used since before recorded history for curing and preventing various ailments.The bioactive chemical constituents and antimicrobial activity of five medicinal plants against S. aureus and E. coli were evaluated. A qualitative phytochemical analysis was performed for the detection of carbohydrates, reducing sugars, saponins, flavonoids, steroids, alkaloids and glycosides.Revealed that M. arvensis and C. frutescens were found to have greater number of secondary metabolites. The ethanolic extract of M. arvensis, C. frutescens and C.aromaticus showed significant antimicrobial activity against S. aureus. Extracts of C. citratus, C. frutescensand C.aromaticus werealso found to be effective against E. coli. The occurrence of these bioactive metabolites in the five medicinal plants may justify their wide usage in traditional medicine.***

Keywords: Phytochemical analysis, Antimicrobial activity, Medicinal plants, Agar well diffusion method, Leaf extract, Zone of inhibition

1. Introduction

Since before recorded history, plants have been used as traditional medicine and remain until now as one of the important resources to combat serious diseases in the world. The discovery of medicinal plants typically resulted from the populace's experience of long and dangerous self-experiments. According to Holiman (1989), the better understanding of a plant-derived medicine has depended on two factors. First, the development of increasing strict criteria of evidence that amedicine really does what it isclaimed to do and secondly, the identification by chemical analysis of the active compound in plant. About 80% individuals from developing countries rely on plant-based preparations used in their traditional medicinal system and as the basic needs for human primary health care (Ellof, 1998). Plants used for traditional medicine contain a wide range of bioactive molecules, making them rich sources of different types of medicine particularly antimicrobial properties (Nair et al.,2005).Mostly, these compounds are secondary metabolites such as alkaloids, flavonoids, steroids, tannins and phenol compounds.

Nowadays, the major problem faced by the world is the multiple drug resistance pathogens (MDR) because of the indiscriminate use of commercial antimicrobial drugs. According to Yalaet al.(2001),the massive use of antibiotics in human therapy resulted to bacteria developing several resistance mechanisms since these microorganisms possess the genetic ability to acquire and transmit resistance to therapeutic agents. This resistance problem poses a demand to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for their potential antimicrobial properties. According to Cunha(2001), antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously minimizing many of the side effects that are often associated with synthetic antimicrobials or antibiotics. There are many published reports on the effectiveness of traditional plants against Gram-positive and Gram-negative microorganisms, and as a result plants are still recognized as the basis for modern medicine to treat infectious diseases (Evans, 2002).

In the present study, five commonly used Surigaonon medicinal plants *Curcuma longa* (Duyao), *Cymbopogoncitratus* (Tangyad), *Coleus aromaticus* (Garabo), *Menthaarvensis* (Helbabuena) and *Capsicum frutescens* (Sili) were assessed for their phytochemical contents and antimicrobial activity of their leaf extracts against *Staphylococcus aureus* (gram positive) and *Eschericia coli* (gram negative) bacteria.

2. Materials and Methods

Collection of plant materials

The fresh and healthy leaves of the selected medicinal plants used for the experiment were *Curcuma longa* (Duyao), *Cymbopogoncitratus* (Tangyad), *Coleus aromaticus* (Garabo),*Menthaarvensis* (Helbabuena) and *Capsicum frutescens* (Sili). The plant materials were collected from July to August 2015 from various areas in Surigao del Sur, Philippines. The plant specimens were identified in Biology Department of Mindanao University of Science and Technology.

Preparation of extracts

The plant materials collected were washed thoroughly and dried in shade and powdered in a mechanical grinder. One hundred grams of each respective plant parts were soaked to 300 mL ethanol and kept at room temperature for 24 hours. The extracts were filtered using whattman filter paper (No.1) and dried at below 45^{O}C for the removal of ethanol for obtaining the dense extract or determining the concentration in mg/mL. The extract was preserved at 2-4 ^{O}C and for further investigation for potential antimicrobial properties.

Phytochemical analysis

The extracts of each medicinal plants were subjected to phytochemical testing to ascertain the presence of

metabolites such as carbohydrates, reducing sugars, saponins, flavonoids, alkaloids and glycosides by following the standard procedures.

Test for Carbohydrate
The plant extract was mixed with few drops of Benedict's reagent and boiled in water bath. The formation of reddish brown precipitate showed the positiveresult for the presence of carbohydrate. (Raman, 2006)

Test for Reducing Sugar
A 0.5 mL of plant extracts was added with 1 mL of distilled water and 5-8 drops of Fehling's solution and was heated over water bath. Formation of brick red precipitate indicates the presence of reducing sugars. (Iyengar, 1995).

Test for Saponins
The plant extract was mixed with water. It was shaken and observed for the formation of froth, which is stable for 15 minutes. This showed a positive result (Raman, 2006).

Test for Flavonoid
The 4 mg of plant extract was treated with 1.5 mL of 50% methanol. The mixture was warmed and was added with magnesium ribbon. To the mixture, 5-6 drops of concentrated hydrochloric acid was added. Formation of red or orange colour indicates the presence of flavonoids. (Siddiqui and Ali, 1997).

Test for Alkaloid
The ethanolic extract of plant was evaporated to dryness. The residue was then heated on a boiling water bath with 2% hydrochloric acid. It was then cooled and the mixture was filtered and treated with a few drops of Meyer's reagent. The presence of turbidity or yellow precipitate showed the presence of alkaloid (Evans, 2002).

Test for Glycoside
The plant extract was treated with few drops of glacial acetic acid and few drops of ferric chloride and mixed. Theconcentrated sulphuric acid was added to the mixture and observed for the formation of two layers. The upper acetic acid layer with a bluish green colour indicated a positive test for glycosides and the lower layer with a reddish brown colour. (Harborne, 2005).

Isolation of pathogens
Two test pathogens *Escherichia coli* and *Staphylococcus aureus* used in this studywere isolated from water samples bystandard methods. Identity of the isolateswas confirmed by morphologicalcharacteristics and conventional biochemical tests (Harley and Prescott, 2002). Purecultures were preserved at 4°C on nutrientAgar.

Determination of antibacterial activity
Antimicrobial activity of five medicinal plant extracts was determined by agar well diffusion method. A 0.1 mL of freshly grown culture of test organisms (106cfu/mL) was aseptically introduced and spread on surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter were made in agar plate with the help of sterile cork-borer. Fifty microliters of different plant extracts were filled in the wells with the help of micro pipette.

Standard reference antibiotics like Chloramphenicol was used as positive controls for the test organisms. Plates were left for some time at 4°C till the extract diffuses in the medium with the lid closed and incubated at 37°C for 24 hr. The plates were observed for zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of the zone ofinhibition against the tested bacterial pathogens. Each assay in this experiment was replicated three times (Jain, 2009; Joshiet al., 2011).

3. Results and Discussion

In this study, gram positive bacterial pathogen *S. aureus*and gram negative pathogen *E. coli* were selected and antimicrobial activity of leaf extracts of plants *Curcuma longa* (Duyao), *Cymbopogoncitratus* (Tangyad), *Coleus aromaticus* (Garabo), *Menthaarvensis* (Helbabuena) and *Capsicum frutescens* (Sili) were evaluated against them. The phytochemical screening of the five medicinal plants revealed the presence of various secondary metabolites like carbohydrates, reducing sugar, saponins, flavonoids, steroids, alkaloids and glycosides some of which have been previously associated with antimicrobial activity (Nweze, 2004). These bioactive metabolites are known to act by different mechanism. Like alkaloids, they have shown to possess both antibacterial andantidiabetic properties (Adeshina et al., 2012). Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Dharet al.,1979). Steroids have been reported to have antibacterial properties (Raquel et al., 2007) and saponin has antimicrobial property which is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowiczet al., 1996). Table 1 revealed that out of 9 metabolites tested *M. arvensis and C. frutescens*had the highest number of metabolites followed by *C.citratus*and the least is C. *longa.* The presence of these metabolites is an indicator that the plants can be a potential source of precursors in the development of synthetic drugs.

Table 1: Phytochemical analysis of Ethanol extracts of Selected Medicinal Plants

Secondary Metabolites	**Medicinal Plants**				
	C. longa (duyao)	*C.citratus* (tangyad)	*C. aromaticus* (garabo)	*M. arvensis* (helbabuena)	*C. frutescens* (sili)
Carbohydrates	+	+	-	+	+
Reducing sugar	+	+	+	+	+
Tannins	-	-	-	-	-
Saponins	+	+	-	+	+
Flavonoids	-	-	+	+	+
Steroids	-	+	+	+	+
Alkaloids	+	+	+	+	+
Anthraquionones	-	-	-	-	-
Glycosides	-	+	+	+	+

(-) absence of secondary metabolites, (+) presence of secondary metabolites done in triplicates

Table 2 revealed that ethanol extracts of *M.arvensis* has highest inhibitory activity against all two test organisms *E. coli* and *S. aureus*. The maximum zone of inhibition (15.537 mm) against *S.aureus* was obtained in *M.arvensis* and the maximum zone ofinhibition (15.253 mm) against *E.coli* was obtained in *C.aromaticus*. The present results confirmed the study of Sugandhi and Meera (2011) that the ethanol extract of *M. arvensis* inhibited the growth of *E. coli* and *S. aureus* including*P. aeruginosa, S. flexineri, K. pneumoniae*pathogens. In addition Subhas et al. (2010) found in their study that *C.aromaticus*showed more inhibition zone in *E.coli*.*C.aromaticus* and *C.frutescens* showed moderate antimicrobial activity while *C.citratus*and *C. longa* extracts showed considerable antimicrobial activity against *S.aureus*(gram positive).For gram negative pathogen *E. coli*, *C.citratus*, *M.arvensis* and *C.frutescens* showed moderate antimicrobial activity and *C. longa* showed less significant in antimicrobial activity.The graph in Figure 1 shows the average diameter of zone inhibition of five medicinal plants against both gram-negative and gram-positive bacteria. Results revealed also that the ranged of inhibition of the five medicinal plant extracts are lower than the range ofof inhibition of Chloramphenicol as standard.Lans, et al. *(*2001) stated that the demonstration of antimicrobial activity against both gram positive and gram negative bacteria by the plants may be indicative of the presence of broad spectrum of antibiotic compounds. Furthermore, the optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of differentcompounds originally in the plant(Bhandarkaret al.,2003).

Table 2: Antimicrobial property of Selected Medicinal Plants against Gram positive and gram negative bacterial species tested by disc diffusion assay Zone of inhibition (mm)

Medicinal Plants	Bacterial Pathogens	
	Staphylococcus aureus (Gram positive) mm	*Escherichia coli* (Gram negative) mm
Curcuma longa (duyao)	7.347±0.560	7.470±0.104
Cymbopogoncitratus (tangyad)	7.633±0.064	14.033±0.115
Coleus aromaticus (garabo)	15.000±0.100	15.253±0.040
Menthaarvensis (helbabuena)	15.537±0.058	13.627±0.248
Capsicum frutescens (sili)	14.970±0.061	11.117±0.196
Chloramphenicol (500 mg)	23.013±0.110	23.960±0.242

Means of zone inhibition ± standard deviation.

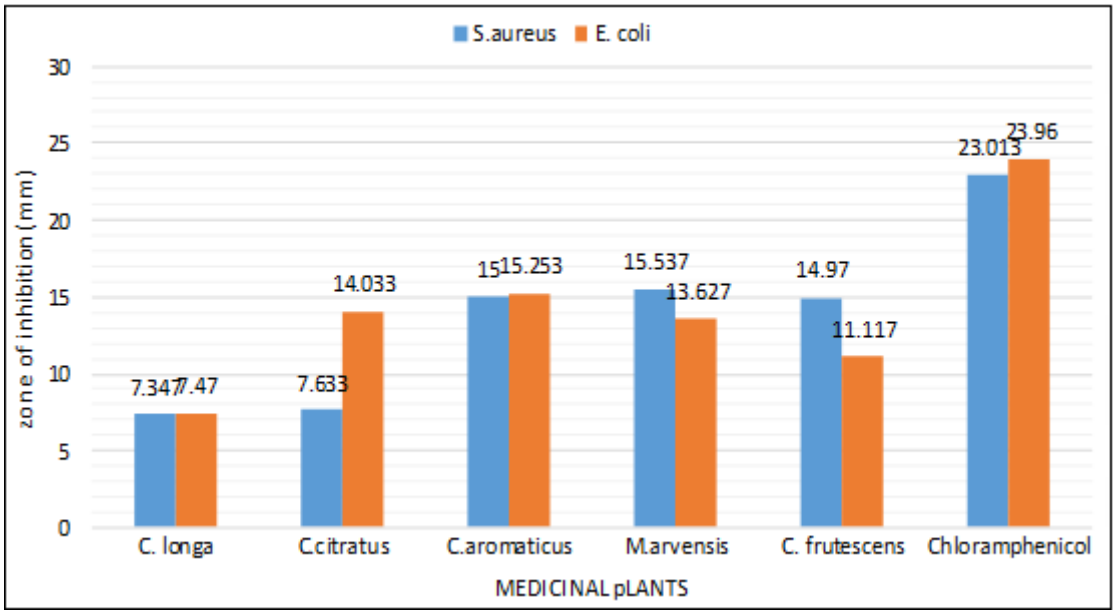

Figure 1: Average diameter of zone inhibition exhibited by the Medicinal Plants against E.coli(Gram-negative) and *S. aureus* (Gram-positive)

In addition, the antimicrobial activity of medicinal plants (Arulmozhi *et al.,* 2007) may be due to the presence of different chemical agents which were classified as bioactive antimicrobial compounds. The results of the present study confirmed the validity of the use of these medicinal plants as traditional medicines and also suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents. Use of herbal medicine is safer compared to synthetic drugs. The study scientifically proves the importance of plant products in development of a potent antibacterial agent. However, further studies must be carried out to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

4. Conclusion

The result of the present study showed that the extract of *Menthaarvensis* which contain the highest number of metabolites, exhibited highest inhibitory activity against two

test organisms *E. coli*(gram negative) and *S. aureus*(gram positive).The high antimicrobial activity of *Menthaarvensis* may be due to the presence of secondary metabolites present in plants that provide the necessary component as antimicrobial agent. All of the extracts in this research exhibited different extent of antimicrobial activity.

References

[1] Adeshina G.O., Kunle O.F., Onaolapo J.O., Ehinmidu J.O., Odama L.E. 2012. Phytochemical and Antibacterial Studies of the Hexane Extract of Alchornea Leaf. Phytochemicals as NutraceuticalsGlobal Approaches to Their Role in Nutrition and Health. pp. 90-96
[2] Arulmozhi, S., Mazumder, P.M., Ashok, P.,Narayanan, L.S. 2007. Pharmacological activities of *Alstoniascholaris*Linn. (Apocynaceae)- A review. *Pharmaco. Rev.*, 1: 163 165.
[3] Cunha, B.A. 2001. Antibiotics side effects. *Med. Clin. North Am.*, 85: 149 185.
[4] Dhar, M.L., Dhar, M.M., Dhawan, B.N., Ray,C. 1979. Screening of Indian plants for biological activity. *Ind. J. Biology.*, 6:232 234.
[5] Ellof, J.N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants?*J. Ethnopharmacol.*, 60: 1 6.
[6] Evans, W.C.,2002. Trease and Evan's Pharmacognosy. 5th ed., Haarcourt Brace and Company, pp 336.
[7] Evans CE, Banso A, Samuel OA*; J Ethno pharmacology*, 80 **2002,** pp. 21–24
[8] Harborne JB. Phytochemical Methods. New Delhi: Springer (India) Pvt.Ltd; 2005. p.17
[9] Harley, J.P., Prescott, L.M. 2002. Laboratory exercises in microbiology. 5th edn, McGraw Hill, New York.
[10] Holiman, A., 1989. Plants in Medicine. Chelsea Physic Garden. The Chelsea Physic Garden Co. Ltd.
[11] Iyengar, M.A. 1995. Study of drugs, 8th edn, Manipal Power Press, Manipal, India.
[12] Jain, P., Bansal, D., Bhasin, P. 2009. Antibacterial activity ofaqueous plant extracts against *Escherichia coli* and *Bacillus subtilis. Drug Invent. Today*, 2(4): 220 222.
[13] Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.,Sharma, D., Subedi, K., Pandey, J., Malla, R. 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants*: Ocimumsanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthesbidentata* (Datiwan) and *Azadirachtaindica* (Neem). *J. Microbiol. Antimicrob.*,3(1)
[14] Nair, R., Kalariya, T., Chanda, S. 2005. Antibacterial activity of some selected Indian medicinal flora.*Turk. J. Biol.*,29: 41 47
[15] Nweze, E.T., J.I. Okafor and O. Njoku, 2004. Antimicrobial Activities of Methanolic extract of Trumeguineesis (Schumm and Thorn) and MorindalucindaBenth used in Nigerian Herb.Medicinal Practice. J. Bio. Res. Biotechnol., 2(1): 34-46.
[16] Raman N. Phytochemical Technique. 2006; New Indian Publishing Agencies: New Delhi p.19*mexicana* L. (Papaveraceae). *CMU J. Nat. Sci.*, 8: 77 84.
[17] Raquel, F. Epand, 2007. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. *BiochimicaetBiophysicaActa*, Pp. 2500 2509.
[18] Siddiqui, A.A., Ali, M. 1997. Practical Pharmaceutical Chemistry. 1st Ed.,CBS Publisher and Distributors, New Delhi, pp 126-131.and Pharmaceutical Sciences, ISSN-0975-1491 Vol 2, Issue 3.
[19] Sugandhi, Rachel Madhuri and BaiMeera. 2011. Antimicrobial Activity of *MenthaArvensis*L. Lamiaceae. Journal of Advanced Laboratory Research in Biology.
[20] Yala D, Merad AS, Mohamedi D, OuarKorich MN. Classification et mode d'action des antibiotiques. Médecine du Maghreb, 2001; 91:5-12.
[21] Zablotowicz, R.M., Hoagland, R.E., Wagner, S.C. 1996. Effect of saponins on the growth and activity of rhizosphere bacteria. *Adv. Exp. Med. Biol.*, 405: 83 1.95.