Chemistry

GC-MS ANALYSIS, PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *Alangium salviifolium* BARK EXTRACTS.

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Abstract

Extracts of *Alangium salviifolium* (L. f.) Wanger in bark have been locally used in Meche Community of Jhapa district by the old and traditional practitioner for the treatment of Diarrhoea, piles and vomiting.etc. Hexane, Dichloromethane, ethylacetate, methanol and 50% aqueous methanol bark extract of *A. salviifolium* were undertaken for the investigation of phytochemical screening and antibacterial activity. The test organisms were diagnostic laboratory isolates: *Escherichia coli* and *Staphylococcus aureus*. The hexane, dichloromethane and ethylacetate extracts showed moderate antibacterial activity against both Gram positive and Gram negative bacteria whereas methanol and 50% methanol extracts did not show antibacterial activity. Preliminary phytochemical analysis of Hexane bark extract revealed the presence of Terpinoids, Quinones, Saponins and dichloromethane bark extract revealed the terpinoids, quinones, saponins and ethylacetate bark extract revealed the quinones and Methanol extraxt revealed the Reducing sugar, glycosides, polyphenol and flavonoids and 50% methanol extract revealed reducing sugar, glycosides, polyphenol and flavonoids. The chromatogram of methanol extract revealed Pentadecanoic acid , 9,12-Octadecadienoic acid , 10-Octadecenoic acid , Tetracosanoic acid, 9-Octadecenamide , Oxalic acid, Z,E-3,13-Octadecadien-1-ol , 11-Tridecen-1-ol and 16-Hentriacontanone peaks detected by GC-MS in which 10-Octadecenoic acid is a major Phytoconstituent.

Keywords: *Alangium salviifolium, phytochemical, antibacterial, GC-MS*

Introduction

In this present era, many Natural product researchers has focused on the plant research in all over the world and a wealth of evidence shows immense potential of Ethno-medicinal plants in various traditional system (Archana *et al*. 2011). Natural product succeed to deliver about 40% of the top 50 drug and about 80% of the commercially available antibiotics are isolated from micro-organisms (Hebert 1989).

Phyto-constituents are the complex mixture of component so they are able to become more effective than chemically synthesized pure compounds. The phyto-constituent present in the plant has physical or chemical effect in nature which has the ability to minimize the growth of pathogenic or saprobic bacteria (Kumar *et al*. 2014).

The main aim of the present study is focused on the GC-MS Analysis, phytochemical screening and antibacterial activity of the *Alangium salviifolium* (L. f.) Wanger barks. *A. salviifolium* belongs to family Alangiaceae having a tremendous therapeutic potential which is not fully utilized. It is commonly known as Asare in Nepali (Jain *et al*. 2005).

Alangium salviifolium has been used by old traditional practitioners in the treatment of treatment of diarrhea, vomiting. The leaves are used to care asthma and reduce rheumatic pains. The roots are used to cure purgative to kill intestinal worms, treatment of piles, hypertension, fever, back pain and blood disorders (Balkrishnan *et al*. 2010).

The bark, root fruits and leaves contain some of the potential chemical constituent which is responsible for the medicinal importance of herbs. It consists of large concentration of alkaloids, flavonoids and verities of phytoconstituent. This plant consist of large number of phytoconstituent such as 1,methyl-1H-pyrimidine-2, 4 dione, 1,2-dehydrotubulosine, alangine, markine etc (Prajapati *et al*. 2003).

Material and Methods

Plant material

The bark of *Alangium salviifolium* (L. f.) Wanger was collected on the basis of ethno-medicinal information provided by the old experienced people of Meche community of eastern parts of Jhapa District. The bark of plant was collected from the Humse - Dumse community forest which lies in the Northern parts of Damak in January 2016.

The plant samples were identified and authenticated by the Senior Botanist of the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu. Voucher specimen (collection number: AS-DB) was deposited at Research Center for Applied Science and Technology, Tribhuvan University.The collected plant materials were washed under tap water in order to remove the contaminants like dust, soil, insect larvae and fungal infected parts were discarded. The cleaned plant sample was shade dried. The dried sample was ground into powder and stored in air tight plastic bag to prepare different extract.

Reagents and chemicals

All the Organic solvents like Methanol, Ethylacetate, Hexane and Dichloromethane and chemicals like Na_2CO_3, $NaNO_2$, NaOH, (Sd. Fine Chemical limited, Mumbai) were of analytical grade and purchased from local vendors and double distilled water was purchased from the local vendor.

Preparation of different extract of *Alangium salviifolium* (L. f.) Wanger bark

The dried and powdered plant material (80g) was extracted successively with hexane, dichloromethane, ethyl acetate and methanol by cold percolation method. The plant material was immersed in the respective solvents for 24 h and the extract was filtered and another fresh solvent was added. The process was repeated 5-6 times until the last extract was nearly colorless. The remaining residue after extraction with methanol was refluxed with 50% aqueous methanol for 2 hours then allowed to cool and filtered. The filtrate was concentrated under rotary evaporator at reduced pressure to obtain solid or semisolid extract. The dried extract was kept in freeze for further analysis.

Phytochemical screening of *Alangium salviifolium* bark

The phytochemical components of the extracts were determined according to standard protocol (Kulkarni and Addepalli, 2010; Lalitha and Jayanthi, 2012; Alamzeb *et al*. 2013). This process involves the selective and successive extraction of the natural phyto-constituents with solvent of increasing polarity. The different phyto-constituent in the various extracts was identified by their specific color reaction with different reagents.

Antibacterial assay

Agar well diffusion method was used in the study of evaluation of antibacterial activity of the crude extract and isolated fraction. Inhibition of the bacterial growth was tested by agar well diffusion method and measured in the form of zone of inhibition (ZOI) given by Dingel *et al.* (1953).

Preparation of working solution

Each of the crude extracts 50 and 100 mg was weighted and transfer in a clean, sterile vial aseptically and dissolved in 1 ml Dimethyl sulphoxide (DMSO).

Collection of standard culture

Control strains of Gram-positive bacteria (*Staphylococcus aureus* ATCC, strain 25923) and Gram-negative bacteria (*Escherichia coli* ATCC, strain 25922) were selected for determining antimicrobial activity of the different extracts. The bacteria were provided by the Department of Microbiology of GoldenGate international College.

GC-MS analysis of methanol extract of *Alangium salviifolium* (L. f.) Wanger bark was done for the identification of chemical composition. Methanol extract was analyzed by GC-MS technique in GCMS-QP2010 Ultra SHIMADZU spectrometer fitted with Rtx-5MS capillary column (30 m x 0.25 mm id., film thickness 0.25 μm). The initial oven temperature was 50°C. The temperature was gradually increased to 160°C at a rate of 20°C/minute. Again the temperature was gradually increased up to 300°C at a rate of 8.00°C/minute and kept at final temperature for 2 minutes. The maximum oven temperature was maintained at 300°C with an equilibrium time of 1 minute.

The ion sources temperature was 200°C while GC interface temperature and inlet temperature was kept at 250°C. Diluted sample (1.0 µL) was injected at 280°C in the splitless mode. Helium was used as a carrier gas with flow rate of 1.0 ml/min.

MS was operated in electron impact mode with ionization energy of 70 eV. Full scan mass spectra were acquired from 40-500 amu. The total run time was 26 minutes. The detected compounds were identified by processing the raw GC-MS data with GC-MS solution software and comparing with NIST 08 library.

Results and Discussion

Table 1 shows the extractive values of *A. salviifolium* bark in different solvents. The percentage yields of the various extracts are shown in table 1. Methanol extract have highest percentage yield.

Table 1. Amount of different extracts obtained from *A. salviifolium* bark.

Extracts	Hexane	CH_2Cl_2	EtOAc	MeOH	50% MeOH
Yield in gram	2.431	3.17	1.838	15.185	7.260
Yield in %	3.0375	3.9625	2.297	18.981	9.075

The table 2 shows results of phytochemical screening of Hexane, dichloromethane, ethylacetate, methanol and 50% methanol extracts of *Gmelina arborea* barks. The results revealed the presence of Terpenoids, Quinones, Saponins, Fatty acid for Hexane extract and dichloromethane extract and quinones for ethylacetate extract and Flavonoids Reducing sugar, Polyphenols Glycosides for methanol and 50% methanol. Table 2 shows the effect of different concentrations of different extract of *A. salviifolium* barks on the bacteria isolates.

The hexane, dichloromethane and ethylacetate extracts showed moderate antibacterial activity against both Gram positive and Gram negative bacteria whereas methanol and 50% methanol extracts did not show antibacterial activity.

Table 2. Phytochemical screening of different extract

A.salviifolium bark extract	Hexane	CH_2Cl_2	EtOAc	MeOH	50% MeOH
Volatile oil	-	-	-	-	-
Alkaloids	-	-	-	-	-
Flavonoids				+	+
Terpenoids	+	+	-	-	-
Glycosides	-	-	-	+	+
Quinones	+	+	+	-	-
Reducing sugar	–	–	–	+	+
Polyphenols	-	-	-	+	+
Saponins	+	+	-	-	-
Fatty acid	+	+	-	-	-
Tannins	-	-	-	-	-

(+) indicates present and (–) indicates absent

Different concentration of different extract of *A. salviifolium* bark showed antibacterial activity. The results of this research show the effective antibacterial activities. The variation in antibacterial activity is due to extraction solvent and test organisms used.

The lower concentration of hexane did not shows any effect against *E. coli* but higher concentration shows positive effect against *E. coli* whereas both concentration of hexane extract shows effect against *S. aureus*. Ethyl acetate extracts showed moderate antibacterial activity against both Gram positive and Gram negative bacteria whereas methanol and 50% methanol extracts did not show antibacterial activity.

Table 2. Effect of different concentrations of different extract of *A. salviifolium* barks on the bacteria isolates.

***A. salviifolium* bark extract**	**Organisms**	**Zone of inhibition (mm)**		**Control**
		50 mg/ml	100mg/ml	
Hexane	*E.coli*	_	11	
	S. aureus	10	12	
CH_2Cl_2	*E.coli*	-	12	
	S. aureus	-	-	
EtOAc	*E.coli*	11	12	
	S. aureus	14	16	
MeOH	*E.coli*	_	_	
	S. aureus	_	_	
50% MeOH	*E.coli*	_	_	
	S. aureus	_	_	
Amoxiciline	*E.coli*			22
10µg/disc	*S. aureus*			36
DMSO 100 µl	*E.coli*			-
	S. aureus			9

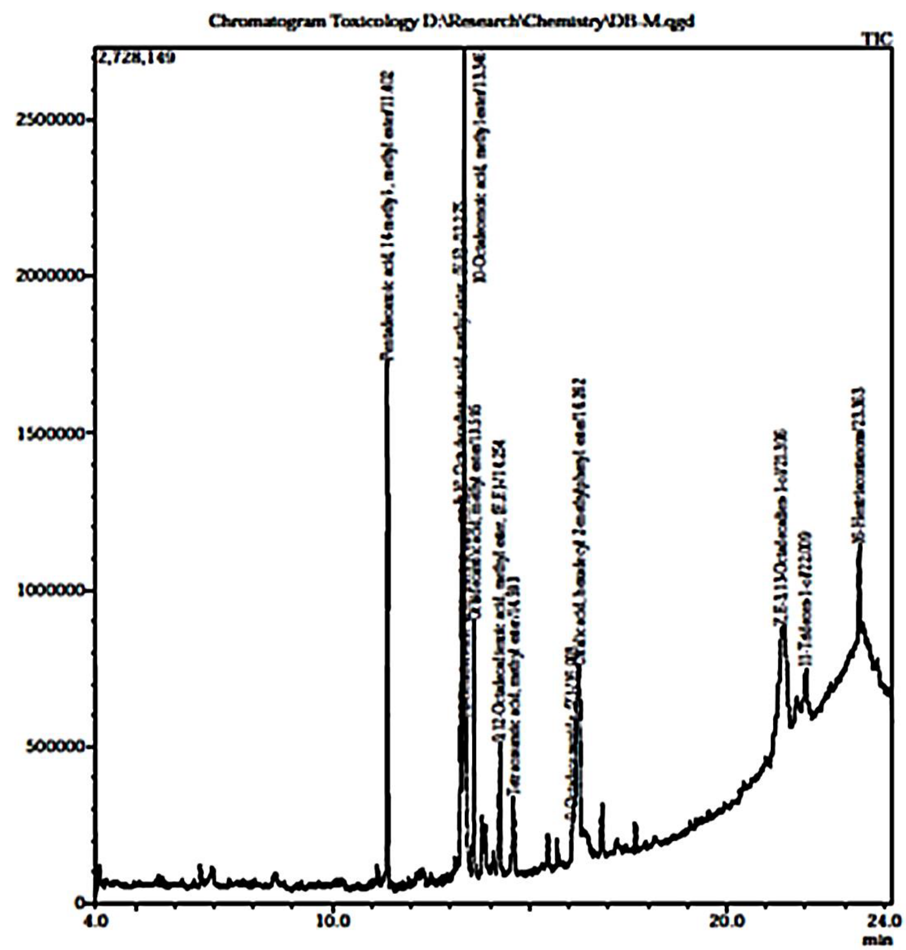

Figure 1. GC-MS analysis of methanol extract of *A. salviifolium* bark for the identification of chemical composition**.** The chromatogram showing Pentadecanoic acid, 9,12-Octadecadienoic acid ,10-Octadecenoic acid , Tetracosanoic acid, 9-Octadecenamide , Oxalic acid, Z,E-3,13-Octadecadien-1-ol , 11-Tridecen-1-ol and 16-Hentriacontanone peaks detected by GC-MS

The figure 1 shows the GC-MS analysis of methanol extract of *A. salviifolium* bark for the identification of chemical composition. The lower concentration of dichloromethane did not shows any effect against *E. coli* but higher concentration shows positive effect against *E. coli* whereas both concentration of dichloromethane extract did not shows effect against *S. aureus.*

The chromatogram of methanol extract of *A. salviifolium* revealed Pentadecanoic acid , 9,12-Octadecadienoic acid , 10-Octadecenoic acid , Tetracosanoic acid, 9-Octadecenamide , Oxalic acid, Z,E-3,13-Octadecadien-1-ol , 11-Tridecen-1-ol and 16-Hentriacontanone peaks detected by GC-MS in which 10-Octadecenoic acid is a major phyto-constituent.

Conclusion

The data obtained from the present study evidently suggested that experimental bacteria treated with ethylacetate extract of *A. salviifolium* showed significant antibacterial activity. From this experimental study it is concluded that *A. salviifolium* which is used in ethnomedicine in Meche community of Jhapa district could be use in primary health care or it could be the source of bioactive compounds for future drug development. These plants could also be use as a valuable functional food ingredient or a source for nutraceutical products in the future which may have possible beneficial implications in human health such as in the treatment various ailments.

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