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Pharmacognostic and Phytochemical Screening of *Vernonia amyggalina Linn* Against Selected Bacterial Strains

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Abstract: In the present study, the active phytocomponents of *Vernonia amyggalina were* revealed using phytochemical analysis. The antimicrobial activity of *Vernonia amyggalina* was studied using well diffusion method. The activity was tested via determination of antibacterial activities of the extracts of *Vernonia amygdalina. Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus* and two yeasts *Candida albicans* and *Saccharomyces cerevisiae* standard bacterial strains were used and the results have been illustrated. In addition, phytochemical screening methods were applied for identifying the major chemical groups in these species. This study provided referential botanical and phytochemical information for correct identification of these leaves. Preliminary phytochemical analysis of extracts revealed the presence of antimicrobial compounds such as alkaloids, flavonoids, tannins, saponins etc.Among the test samples, ethyl acetate extract showed pronounced antimicrobial activity.

Key words: Antibacterial · Antifungal · *Vernonia amyggalina Linn*

INTRODUCTION

New drug discoveries have shifted attention from synthetic models and compounds to natural products of plants origin. This is because scientists now believe that drug leads/hit molecule discovery would be more probable in plant and other natural sources like marine and animals which are yet to be fully explored. This drift has promoted, in recent time, researches in plants considered to be of little or no economic or ecologic significance. Extracts of leaves of *Vernonia amyggalina Linn* have been found to possess antimalarial activity against plasmodium [1, 2] and activity against sexually transmitted diseases [3]. Chewing sticks of *V. amygdalina* has been found to have antibacterial activity [4] and water soluble anti cancer agents have been discovered from the plant [5]. This study was undertaken to determine the antimicrobial activity of *Vernonia amygdalina* leave extract.

MATERIALS AND METHODS

Leaves were collected from Deveri Distt. Sagar [M.P]. Fresh leaves materials were shade dried and homogenized to a fine powder. The identification of the plant was carried out at the Department of Pharmaceutical Sciences Dr. H.S. Gaur University Sagar [M.P.].

Plant Extraction: The powdered medicinal plant material was taken and subjected to successive solvent extraction in the increasing order of polarity i.e. from non polar to high polar. The solvents used are Acetone, Methanol, Ethanol and Cold aqueous. For above solvent extraction 50g of powdered plant material of *Vernonia amyggalina Linn* was mixed with 250 ml of Petroleum ether and subjected to occasional shaking for 24 hrs. The extracts were filtered through Muslin cloth and extracts were concentrated by evaporation at room temperature until the solvent gets evaporated completely. After complete drying the above said residues were extracted with all the other solvents separately. Then extracted materials lyophilized by occasional shaking for 24 hrs.

Preparation of Non-Polar and Polar Extracts: A 250g dry powder of *Vernonia amyggalina Linn* was sequentially extracted with dichloromethane and methanol using the Soxhlet apparatus on the water bath for 12 h each [6].

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Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction. A 10g amount of pulverized fruits was separately soaked in 100ml of acetone, ethanol, methanol (100% each) and cold sterile distilled water for 24h. Also the same amount (i.e. 10g) of pulverized fruits was immersed in 100 ml of hot sterile distilled water and allowed to stand for 30min on a water bath with occasional shaking and kept undisturbed for 24h. Each of the mixtures was carefully filtered using filter paper (Whatman No. 41) and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210) at 40°C. The final concentrated extracts were stored at -5°C in labeled sterile bottles and kept as aliquots until further evaluation. Another 250 g of powdered sample of the herb was extracted by soaking in 1 L double distilled water in a round bottom flask, stirred for about 6 min, closed tight using a rubber cork and left overnight at room temperature. Thereafter, the solution was filtered using filter paper (Whatman No. 41) and extract was freeze dried and carefully stored at -5°C in labeled sterile bottles.

Phytochemical Screening: Phytochemical screening of plant extracts was carried out qualitatively for the presence of terpenoids, steroids, tannins, flavonoids, amino acids, glycosides, saponins and alkaloids [6].

Determination of Alkaloids: A measured weight of the sample was dispensed into 45 % acetic acid solution in methanol to form a ratio of 1:20. The mixture was allowed to stand for 4 hours at 35°C. It was later filtered with filter paper and the filtrate was treated with drop wise addition of aqueous NH_4OH until the alkaloid was precipitated, this was washed with 10% ammonia solution and dried in the oven at 80°C.

Determination of Flavonoids: A 5g of the sample was boiled in 50ml of 1M HCl solution for 30 minutes under reflux. It was allowed to cool, then filtered through filter paper and the filtrate was treated with equal volume of ethyl acetate.

Determination of Tannin: A 5g portion of the sample was dispensed in 50ml of distilled water and mixed properly. This was allowed to stand for 30 minutes at 28°C before it was filtered. 2ml of the plant extract was dispensed into a 50 ml volumetric flask. Similarly, 2ml standard solution and 2 ml of distilled water were put in separate volumetric flask. The reagent was added to each of the flask and 2.5 ml of saturated Na_2CO_3 solution was also added, the total content of the flask was made up to 50ml with distilled water and incubated at 28°C for 90 minutes. A spectrophotometer set at 260nm wavelength was used to measure the respective absorbance using the reagent blank to calibrate the instrument.

Determination of Steroid: A measured weight of the sample was dispensed in 100ml freshly distilled water and homogenized in laboratory blender. This was filtered and was eluted with normal ammonium hydroxide solution (pH 9.6). 2ml of the eluate was put into the test tube and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of concentrated H_2SO_4 were added to cool. Standard sterol solution was prepared and spectrophotometer at 254 nm was used to measure the absorbance.

Thin Layer Chromatography: Successive extractions with hexane and chloroform were carried out on the pulverized sample by maceration for 24hrs. The extracts were filtered and concentrated on a rotary evaporator. Each concentrated extract was spotted on a normal phase plate previously activated at 50°C for 5 hrs, using a capillary tube. The plate was developed using mobile phase of hexane-chloroform (11:1) for hexane extract.

Screening for Antibacterial Activity

Bacterial Media (Muller Hinton Media): A 36g of Muller Hinton Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into Petri dishes. The solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

Fungal Media (Potato Dextrose Sugar): A 200g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

Agar Well Diffusion Method: The agar disc diffusion method was employed for the determination of antibacterial activities of the extracts of *Vernonia*

amyggalina Linn. *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus* and two yeasts *Candida albicans* and *Saccharomyces cerevisiae* standard bacterial strains were used. All bacterial cultures were first grown on nutrient agar plates at 37°C for 24 h. The microorganisms were subcultured on the specific media recommended for different microorganisms such as Brain heart infusion agar (*S. mutans*), Nutrient agar (*S. aureus*), Lactobacillus MRS agar (*L. acidophilus*), Malt yeast agar (*C. albicans* and *S. cerevisiae*) and incubated aerobically at 37°C. Identification of all the strains was confirmed by standard biochemical and staining methods [7-9]. Few colonies (2 to 3) of similar morphology of the respective bacteria were transferred to a liquid medium (Mueller Hinton Broth) and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the Mueller Hinton plates. The dried plant extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.45 mm membrane filter. Sterile filter paper discs (5 mm) (Whatman no. 1) were punched and impregnated with 10 µl of the DCM, MeOH and aqueous extracts and allowed to dry at room temperature. These were placed on the Mueller-Hinton agar plates inoculated with the test strains. The plates were then allowed to stay for 1 h at room temperature and finally incubated at 37°C for 24 h. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone (mm) formed around the disc. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Ciprofloxacin (30 µg) and Gentamycin (30 µg) were used as positive controls while 10% DMSO was taken as negative control.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the crude extracts of *Vernonia amygdalina* was determined by agar dilution method (EUCAST, 2000). The growth media, Mueller-Hinton agar (MHA) was first prepared and sterilized by autoclaving. The sterilized MHA was allowed to cool to 50 °C and 18 ml each of the molten agar was added to test tubes which contained 1 ml of different concentrations of the test crude extracts (150 to 300 µg/ml). The mixture of the media and the crude extract were thoroughly mixed and poured onto pre-labeled sterile petri-dishes on a level surface. Additional petri-dishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the respective bacteria. The plates were then set at room temperature and dried. The suspensions of the respective bacteria (corresponding to 10^8 CFU/ml) were inoculated onto the series of agar plates. The plates were then incubated at 37°C for 24 h. Experiments were performed in duplicate and MIC values expressed as the lowest concentration of the plant extracts that produced complete suppression of colony of respective bacteria.

RESULTS AND DISCUSSION

The results of antimicrobial activity of the extracts of *Vernonia amygdalina* by agar well diffusion method have been shown in Tables 1 and 2. From the data presented in the Table 1, it is evident that all the five extracts of *S.mukorossi* i.e. hot aqueous, cold aqueous, acetonic, methanolic and ethanolic showed antimicrobial inhibitory activity against only one of the five tested microorganism *S. cerevisiae*, with the mean diameter of the highest zone of inhibition being 19.61mm. *S. cerevisiae* survived up to 16.1mg/ml in the acetonic extract. Although no inhibitory activity of *S. mukorossi* was shown against *S. mutans, S. aureus, L. acidophilus* and *C. albicans*, the antifungal activity was much higher than the control ciprofloxacin and Gentamycin with the mean diameter being 18.94mm), thus *S. mukorossi* possesses very good antifungal properties against *S.cerevisiae*. All the five tested extracts of Vernonia amygdalina as presented in Table 1 showed antimicrobial activity against *S.mutans* with the mean diameter of the highest zone of inhibition being 22.50mm and an MIC of 90 mg/ml (Table 1), *L. acidophilus, C. albicans* and *S. cerevisiae* did not show any inhibitory activity when assayed against *Vernonia amygdalina* extracts. Thus *Vernonia amygdalina* possesses good antibacterial activity and antifungal activity. The excellent activity of *Vernonia amyggalina Linn* against *S. aureus* showed a very good potential of *Vernonia amyggalina Linn* to treat the diseases caused by *S.aureus*. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

Table 1: Antimicrobial activity of extracts of *Vernonia amygdalina* causing microorganisms determined by agar well diffusion method on specific media for each test microorganism

	Diameter of inhibition zones (mm) *Vernonia amygdalina*				
Vernonia amygdalina (mg/ml)	*S.m.*	*S.a.*	*L.a.*	*C.a.*	*S.c.*
Acetone	-	-	18.54±0.22	-	-
Methanol	14.54±19	22.50±0.2	-	-	-
Ethanol	29.32±0.15	11.31±0.32	-	-	16.4±2
Hot aqueous	33.98±1	19.61±0.33	19.65±0.57	-	-
Cold aqueous	34.66±0.16	19±0.32	-	-	15.43±0.2
ciprofloxacin	31.14±0.25	18.66±0.51	21.65±0.33	nt	nt
Gentamycin	Nt	Nt	Nt	10.5±02	14.94±15
DMSO	-	-	-	-	-

(-) = no activity, nt = not tested, *S.m. = Streptococcus mutans, S.a. = Staphylococcus aureus, L.a. = Lactobacillus acidophilus, C.a. = Candida albicans, S.c. = Saccharomyces cerevisiae,* * Values, including diameter of the well (6 mm), are means of three replicates, † ± Standard deviation

Table 2: MIC of extracts of *Vernonia amygdalina* on specific media for each microorganism, determined by modified agar well diffusion method

	MIC (mg/ml)				
Vernonia amygdalina	Acetone	Methanol	Ethanol	Hot water	Cold water
Streptococcus mutans	85	66	75	55	95
Staphylococcus aureus	16.5	-	-	44.23	32.65

Table 3: Phytochemical Screening of Extract of *Vernonia amygdalina*

S. No	Tests	Leaves of *Mimosa pudica*
1	Terpenoids	+
2	Flavonoids	+
3	Steroids	-
4	Anthroquinone	-
5	Glycosides	+
6	Alkaloids	-
7	Tannins	+
8	Saponins	+

+ Present - Absent

In Conclusion, the *Vernonia amygdalina* extracts have good antibacterial activity against Gram positive and negative organisms. It also possessed strong antifungal activity against *Candida* and other tested fungi. The findings of the present research may lead to the development of natural antimicrobial agents. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine.

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REFERENCES

1. Masaba, S.C., 2000. The anti malarial activity of *Vernonia amygdalina*. Trans. Soc. Trop. Med. Hyg., 94(6): 699-695.
2. Abosi, A.O. and B.H. Roseroka, 2003. *In vivo* anti malarial activity of *Vernonia amygdalina*. British J. Biomedical Sci., 60(2): 89-91.
3. Kambizi, L. and A.J. Afolayan, 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol., 77(1): 5-9.
4. Taiwo, O., H.X. Xu and S.F. Lee, 1999. Antibacterial activities of extracts from Nigerian chewing sticks. Phytother Res., 13(8): 675-679.
5. Izevbigie, E.B., 2003. Discovery of water soluble anticancer agents (edodites) from a vegetable found in Benin City. Nigeria Exp. Biol. Med., 228(3): 293-298.

6. Harborne, J.B., 1998. Phytochemical methods: A guide to modern techniques on plant analysis, third edition. Kluwer Academic Publishers, United Kingdom.
7. Aneja, K.R., 2003. Experiments in Microbiology Plant Pathology and Biotechnology, 4th ed., New Age International Publishers, New Delhi.
8. Benson, H.J., 2004. Microbiological Applications: Laboratory Manual in General Microbiology, McGraw Hill Publication, USA.
9. Cappuccino, J.G. and N. Sherman, 1995. Microbiology Lab Manual, Benjamin-Cummings Publishing Company, USA.