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ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS FROM LEAVES OF A SPECIFIC MEDICINAL PLANT(*Senna obtusifolia*)

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ABSTRACT

This study was carried out to evaluate antimicrobial activity of the crude methanolic extract and its different partitionates i.e. petroleum ether (PEP), dichloromethane (DCM), ethyl acetate (EtOAc) and aqueous (AQP) soluble fractions of leaves of *S. obtusifolia* against some pathogenic bacteria. The powdered leaf of *S. obtusifolia* was extracted with methanol. The ethyl acetate extract exhibited the highest inhibition against microbial growth having zone of inhibition 8 - 20 mm. The maximum zone of inhibition produced by EtOAc was found to be 20 mm against *Sh. dysenteriae* followed by 12 mm against *Escherichia coli*. This partitionate also showed antibacterial activity against *Bacillus cereus*, *B. subtilis* and *B. megaterium* (having zone of inhibition of 11-8 mm) and *Staphylococcus aureus* and *Sarcinalutea* (having zone of inhibition of 9 mm and 11 mm). The extractive also showed antifungal activity against *Sacharomycescerevacae* (6 mm), *Candida albicans* (12mm) and *Aspergillusniger* (7mm). The dichloromethane soluble fraction (AQP) also exhibited mild to moderate inhibition against microbial growth having zone of inhibition 4 - 12 mm. The maximum zone of inhibition produced by DCNSF was found to be 12 mm against *Sarcinalutea*.

KEYWORDS: Extract, Extraction, Zone of inhibition, Antimicrobial activities, Partitionate, Fabaceae.

INTRODUCTION

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents(Wadood *et al*, 2013a). Primary constituents have

chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities.

Bangladesh is a country considered to be rich in MPs genetic resources by virtue ofits favorable agro-climatic condition andseasonal diversity. About 5 000 species of phanerogams and pteridophytes grows inthe country's forests, wetlands, farms andeven roadside as indigenous, naturally occurring or cultivated plants. Of these, more than a thousand have been claimedto possess medicinal or curative properties(Anisuzzaman *et al*, 2015; Haque *et al*, 2007).

Description of *S. obtusifolia (L)*

Senna includes herbs, shrubs, and trees. The leaves are pinnate with opposite paired leaflets. The inflorescences are racemes at the ends of branches or emerging from the leaf axils. The flower has five sepals and five usually yellow petals. The stamens may be different sizes, and some are staminodes. The fruit is a legume pod containing several seeds.(Wu *et al*, 2010).

Taxonomic hierarchy

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae/Leguminosae – Pea family
Genus	*Senna Mill. – senna*
Species	*Senna obtusifolia* (L.)

Scientific name : *Senna obtusifolia (L.)*

Synonym : *Cassia obtusifolia,*

General Botanical Data

Habitat: terrestrial

Flower petal color: orange, yellow.

Leaf type: the leaves are compound (made up of two or more discrete leaflets

Leaf arrangement: alternate: there is one leaf per node along the stem

Leaf blade edges: the edge of the leaf blade is entire (has no teeth or lobes)

Flower symmetry: there is no way to evenly divide the flower (the flower is asymmetrical)

Number of sepals, petals or tepals: there are five petals, sepals, or tepals in the flower

Fusion of sepals and petals

- Both the petals and sepals are separate and not fused
- The petals or the sepals are fused into a cup or tube

Stamen number: 7

Fruit type (general): The fruit is dry and splits open when ripe.

Habitat

S. obtusifolia or Sicklepod and senna's are weedy in many tropical countries around the world and are thought to be native to America. Sicklepod and foetidsenna occur predominantly in pasture and sugar cane along the tropical east coast of Queensland (from Sarina to the tip of Cape York) and the Top End of the Northern Territory. Hairy senna is a perennial weed of pastures and rainforests along coastal Queensland and northern New South Wales. Dense infestations occur north of Mackay and southwest of Ingham as well as some parts of the Atherton tableland. Sicklepod prefers well drained, fertile soils and is well suited to cleared coastal forest country.(Dunlop *et al*, 2006).

MATERIALS AND METHODS

Senna obtusifolia, belonging to the family Fabaceae, was investigated for its chemical constituents.

Collection and preparation of the plant material

The leaves of S. obtusifolia were collected from Dhaka in December, 2014. The plant was identified at the Bangladesh National Herbarium, when a voucher specimen has been deposited for this collection. After proper washing, the leaf was sun dried for several days. The dried plant was then ground to a coarse powder using high capacity grinding machine.

Extraction of the plant material

400 gm of the powdered material was taken in a clean, amber colored bottle (3 liters) and soaked in 2 liters of methanol. The container with its content was kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton plug and finally with a Whatman No.1 filters paper. The volume of the filtrate was then reduced using a Buchii Rotavapour at low temperature and pressure. The weight of the crude extract was 40 gm.

Solvent-solvent partition of crude extract

Solvent-solvent partitioning was done using the protocol designed by Kupchan and modified by Van Wagenen. The crude extract (5 gm) was dissolved in 10% aqueous methanol. It was extracted with n-hexane, then with carbon tetrachloride and finally with dichloromethane.

All the four fractions were evaporated to dryness (Table 1) and were used for further analysis.

Table 1: Amount of partitionates obtained from (40 gm) methanolic extract.

Plant part	Sample code	Fraction	Weight (gm)
Leaves	PEP	Petroleum ether soluble fraction	5.7
	CTP	Carbon tetrachloride soluble fraction	4.2
	CHP	Chloroform soluble fraction	2.4
	AQP	Aqueous soluble fraction	12.6

Methods of Antimicrobial Test

Worldwide, infectious disease is one of main causes of death accounting for approximatelyone-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics indeveloping nations, but what may be remarkable is that infectious disease mortality rates areactually increasing in developed countries, such as the United States. Death from infectiousdisease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, an increase of58%. It is estimated that infectious disease is the underlying cause of death in 8% of thedeaths occurring in the US (Pinner *et al*., 1996). This is alarming given that it was oncebelieved that we would eliminate infectious disease by the end of the millenium.

Theincreases are attributed to increases in respiratory tract infections and HIV/AIDS. Othercontributing factors are an increase in antibiotic resistance in nosicomial and communityacquired infections. Furthermore, the most dramatic increases are occurring in the 25–44 yearold age group (Pinner *et al*., 1996). These negative health trends call for a renewed interest in infectious disease in the medicaland public health communities and renewed strategies on treatment and prevention. It is thislast solution that would encompass the development of new antimicrobials (Fauci, 1998). The antimicrobial screening which is the first stage of antimicrobial drug research isperformed to ascertain the susceptibility of various fungi and bacteria to any agent. This testmeasures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth.This ability may be estimated by any of the following three methods (Ayafor, 1972).

- Disc diffusion method

- Serial dilution method
- Bioautographic method

Principle of disc diffusion method

In this classical method, on nutrient agar medium uniformly seeded with the test microorganisms dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed. Antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Standard antibiotic (ciprofloxacin) discs and blank discs are used as positive and negative control.

To allow maximum diffusion of the test materials to surrounding media these plates are kept at low temperature (4°C) for 16 to 24 hours (Barry, 1976). For optimum growth of the organisms the plates are then inverted and incubated at 37°C for 24 hours.

The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The diameter of zone of inhibition expressed in millimeter is then measured to determine antimicrobial activity of the test agent (Barry, 1976; Bayer *et al.*, 1966).

In the present study the crude extracts as well as fractions were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required (Bayer *et al.*, 1966).

Experimental procedure

The experiment was done by following the method illustrated by Bayer *et al.*, 1966

Test organisms

Table 2: List of bacteria and fungi used in antimicrobial screening.

Gram positive Bacteria	Gram negative Bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Salmonella* Paratyphi	*Aspergillusniger*
Bacillus subtilis	*Salmonella* Typhi	*Sacharomycescerevacae*
Sarcinalutea	*Shigellaboydii*	
Staphylococcus aureus	*Shigelladysenteriae*	
	Pseudomonas aeruginosa	
	Vibrio mimicus	
	Vibrio parahemolyticus	

TEST MATERIALS

Table 3: List of Test materials.

Plant part	Sample code	Test Sample	Dose µg/disc	Required amount for 20 disc (mg)
Leaves of *S. obtusifolia*	ME	Methanolic extract	400	8.0
	PEP	Petroleum ether partitionate	400	8.0
	DCM	Dichloromethane	400	8.0
	EtOAc	Ethyl acetate	400	8.0
	AQP	Aqueous partitionate	400	8.0

Preparation of the medium

To prepare required volume of this medium, calculated amount of each of the constituentswas taken in a conical flask and distilled water was added to it to make the required volume.The contents were heated in a water bath to make a clear solution. The pH (at 250C) wasadjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred inscrew cap test tubes to prepare plates and slants respectively. The test tubes were then cappedand sterilized by autoclaving at 15-lbs. pressure at 1210C for 20 minutes. The slants were usedfor making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

Sterilization procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 1210C anda pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from thepure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. Theinoculated strains were then incubated for 24 hours at 370C for their optimum growth. Thesefresh cultures were used for the sensitivity test.

Preparation of the test plate

The test organisms were transferred from the subculture to the test tubes containing about 10ml of melted and sterilized agar medium with the help of a sterilized transfer loop in

anaseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assurehomogenous distribution of the test organisms in the media.

Diffusion and incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 40C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 370C for 24 hours.

Determination of the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

RESULT

The Methanolic extract of leaves of *S. obtusifolia*(ME) and different partitionates i.e. Petroleum ether (PEP), carbon tetrachloride (CTP), chloroform (CHP) and aqueous (AQP) soluble partitionate of the methanolic extract of whole plant of were subjected to antimicrobial screening with a concentration of 400 µg/disc in every case. The results are given in the Table 4.

Table 4: Antimicrobial activity of test samples of *S. obtusifolia.*

Test microorganisms	**Diameter of zone of inhibition (mm)**					
	ME	PEP	DCM	EtOAc	AQP	Ciprofloxacin
Gram positive bacteria						
Bacillus cereus	7	0	4	8	0	40
B. megaterium	0	7	4	8	0	45
B. subtilis	7	5	10	11	0	35
Staphylococcus aureus	0	0	10	9	0	43
Sarcinalutea	0	7	12	11	0	39
Gram negative bacteria						
Escherichia coli	0	0	9	12	0	38
Pseudomonas aeruginosa	0	0	0	0	0	42

Salmonella Paratyphi	9	0	10	9	7	30
S. Typhi	0	0	7	10	0	35
Shigellaboydii	0	0	0	0	0	38
Sh. dysenteriae	0	0	0	20	12	35
Vibrio mimicus	7	0	9	8	0	35
V. parahemolyticus	0	0	0	0	0	35
Fungi						
Candida albicans	0	0	0	12	0	38
Aspergillusniger	7	0	0	7	0	35
Sacharomycescerevacae	9	0	0	6	10	38

DISCUSSION

The ethyl acetate extract exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 8 mm to 20 mm. The maximum zone of inhibition produced by EtOAcP was found to be 20 mm against *Sh. Dysenteriae* followed by 12 mm against *Escherichia coli*. This partitionate also showed moderate antibacterial activity against, *Bacilluscereus, B. subtilisand Bacillus megaterium* (having zone of inhibition of 11 mm and 8 mm) and *Staphylococcusaureus*and *Sarcinalutea*(having zone of inhibition of 9 mm and 11) and moderate antifungal activity against *Sacharomycescerevacae*(6mm) and *Candidaalbicans*(12mm) and *Aspergillusniger*(7mm) The dichloromethane soluble fraction (AQP) also exhibited some inhibition against microbial growth having zone of inhibition ranged from 4 mm to 12 mm. The maximum zone of inhibition produced by DCNSF was found to be 12 mm against *Sarcinalutea. This partitionate showed moderate antifungal activity against Bacillus cereus, B. megaterium, B. subtilis,* Staphylococcus aureus Salmonella Paratyphi and Vibrio mimicus. The Methanolic extract soluble fraction exhibited antimicrobial activity against *Bacillus cereus, B. subtilis, Salmonella Paratyphi,* and *Vibrio mimicus.*

CONCLUSION

Test materials of *S. obtusifolia* were involved in several biological screening for antimicrobial activities where different fractionates showed biological activities that were statistically evaluated. And antimicrobial activities of test materials ofthe plant extractives were significant. Therefore, considering the potential bioactivity, the plant materials can be further studied extensively to find out their unexplored efficacy and to rationalize their uses as traditional medicines.

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