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Extraction and characterization of secondary metabolites from Wild Senna and analyse it's *in vitro* antimicrobial activity for pharmacological applications

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Abstract

The Asteraceae family stands out as one of the largest and most diverse flowering plant families globally, encompassing over 1600 genera and 2500 species. The importance of weeds in pharmacology encompasses a range of valuable contributions to the field of research. Medicinal plants play a crucial role in the pharmaceutical industry, serving as valuable sources of compounds that serve as precursors for drug development. This research delves into the preliminary phytochemical analysis revealing its richness in phenolic and flavonoid secondary metabolites, with the presence of tannins exclusively in its crude methanolic extract. Through qualitative phytochemical analysis, the research has identified the presence of various bioactive compounds such as Phenols, Flavonoids, Terpenoids, Alkaloids, Tannins, Steroids, Carbohydrates, Glycosides, Amino Acids, and Proteins. Weeds constitute a diverse group of plants, and their various species often contain a wide array of chemical compounds. These bioactive compounds can be explored for their pharmacological effects, including anti-inflammatory, antimicrobial, antiviral, and antioxidant activities. Weeds can serve as precursors for the synthesis of pharmaceutical drugs. Understanding the chemical composition of weeds allows researchers to identify and extract compounds that may be used as starting materials or inspiration for drug development. Weeds have been used in traditional medicine by various cultures for centuries. The collective pharmacological potential of its diverse members underscores the importance of further research and exploration of this plant family for its potential contributions to preventive and therapeutic applications in human health. Utilizing weeds as a sustainable resource aligns with the growing emphasis on environmentally friendly and economically viable drug discovery. This article consolidates current knowledge on the medicinal properties and traditional uses of these plants, shedding light on their wide-ranging pharmacological actions including anti-diarrhoeal, antimicrobial, antihypertensive, anti-inflammatory, analgesic, and anti-plasmodial activities.

Keywords: *Senna obtusifolia*; Phytochemical; secondary metabolite; spectroscopy; Pharmacological activities; antimicrobial; healthcare.

Introduction

Therapeutic plants are basic assets for treating a wide extend of ailments due to their capacity to address medicine resistance issues in microbes[1]. Wild senna (*Senna obtusifolia*) may be a plant species having a place to the Fabaceae family, commonly found in dry and semiarid locale of Asia and Africa. It has been customarily utilized in different restorative hones for its purgative properties due to the nearness of anthraquinone glycoside, fundamentally sennoside. In any case, later investigate has uncovered a broader range of pharmacological exercises related with it auxiliary metabolites, counting antimicrobial properties. Since conventional pharmaceutical takes an all-encompassing approach and employments an assortment of treatments to analyse, anticipate and keep up add up to well- being, its financial noteworthiness has developed essentially. This technique incorporates the utilize of physical methods, otherworldly mending, creature and mineral- based treatment, and plant-based remedies. The pharmacological potential is enormously improved by these auxiliary metabolites, which incorporate phenolic substances, flavonoids, and alkaloids. *Senna obtusifolia* metabolite capacity to combat clinically critical malady with antibacterial properties highlights their significance for pharmacological inquire about and medicine development[2]. Therapeutic plants have bioactive compound which are used for curing of different human illness conjointly play a critical part in mending. Phytochemicals have two categorise, essential and auxiliary constituent[3].

Wild senna contains a diverse array of secondary metabolites, including anthraquinone, flavonoid, saponins, tannins and alkaloids, Anthraquinone are the most studied class of compound due to their laxative and antimicrobial properties. The major anthraquinones found in Wild senna include sennoside, Rhein, aloe- emodin, and chrysophanol. Flavonoids, such as kaempferol and quercetin derivatives, contribute to the plant's antioxidant and anti-inflammatory activities. Saponins exhibit cytotoxic effect against cancer cells and antimicrobial activity against various pathogens. Tannins possess astringent properties and are implicated in wound healing diarrhoea

management. *Senna obtusifolia* is a commercial plant with several domestic and foreign applications in the food, feed, medicinal, gum, paper, and textile sectors[4].

The antimicrobial potential of Wild Senna extracts and isolated compound has been extensively investigated against a wide range of microbial pathogens, including bacteria, fungi, and parasites. *In vitro* studies have demonstrated significant inhibitory effects of Wild Senna extracts against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, *Gram- negative* bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* as well as fungal pathogens like *Candida albicans* and *Aspergillus Niger*. The antimicrobial activity is attributed to the presence of bioactive compound such as anthraquinones, flavonoids and saponins which disrupt microbial cell membrane, inhibit enzyme activity, and interfere with nucleic acid synthesis.

Despite the promising pharmacological potential of Wild Senna, several challenges remain to be addressed. Standardization of extraction methods and quality control measures are essential to ensure consistency and reproducibility of bioactive constituents in herbal preparation, Furthermore, pharmacokinetic studies are needed to elucidate the absorption, distribution, metabolism and excretion of Wild Senna secondary metabolites *In vivo*. Moreover, effects of solvents on synergetic interaction between Wild Senna secondary metabolites is needed to study their pharmacological activities.

Materials and Methods

Sample Collection and Preparation: Wild Senna (*Senna obtusifolia*) plants were collected from Amity University Chhattisgarh Campus, ensuring they were free from any contamination or damage. The collected plant material was carefully cleaned, washed and air – dried to remove any impurities. The dried plant material was ground into a fine powder using a grinder and stored in airtight containers to preserve its bioactive properties (Figure-1).

Extraction of Secondary Metabolites: Various solvents were employed to extract secondary metabolites from Wild Senna. This included solvent extraction using organic solvent such as ethanol, methanol and ethyl acetate. Each extraction method was optimized to ensure maximum yield of bioactive compound[3].

Phytochemical Analysis: Phytochemical encompass carbohydrates, lipid, phenolics, terpenoids, alkaloids and other nitrogen-containing compound. Phytochemical shelter is effective for searching bioactive compound that can be employed to create therapeutic medication. As in our work phytochemical were tested for the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins, phenols, saponins, anthraquinone, and cholesterol in different solvent extract of *Senna obtusifolia*. Sample 1- distilled water, Sample 2-acetone, Sample 3- methanol, Sample 4- ethanol.

Test of Alkaloids: Mayer's Test: Took 6ml of plant extract in a test tube and add 1% of HCl in steam bath and then sample and then add the 1ml of Mayer's reagent (mixture of mercuric chloride and potassium iodide) presence of milkiness shows the existence of alkaloids. This test confirmed the presence of alkaloids[5].

Dragendroff's Test: One portion of the filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids[6].

Bioluminescence and UV-Induced Fluorescence: Examined plant response to stress using bioluminescence and UV-introduced fluorescence before visible symptoms appeared. Recorded response remotely and rapidly due to the non-invasive nature of the technique.

Test of Flavonoids: Took 2ml of each extract and added a few drops of 20% sodium hydroxide. Observed the formation of an intense yellow colour. Added a few drops of 70% dilute hydrochloric acid to the mixture. Observed the disappearance of the yellow colour, indicating the presence of flavonoids in the sample extract[6].

(A) (B) (C)

Figure-1: Collection of plant samples (A) Raw Form; (B) Washed Leaves; (C) Dried form of leaves.

Test of Terpenoids: Took 1 ml of each solvent and added 0.5ml of chloroform. Added a few drops of concentrated sulfuric acid to the mixture. Observed the formation of a reddish- brown precipitate, indicating the presence of terpenoids in the extrac[7].

Test of Steroids: Shook 2 mg of dry extract with chloroform added sulfuric acid slowly to the chloroform layer. Observed for the formation of a red coloured and a bilayer, indicating the presence of steroids in the extract[7].

Test of Glycosides: Dissolved 0.5mg of bark extract in 1 ml of water. Added aqueous NaOH solution to the mixture. Observed the formation of a yellow colour, indicating the presence of glycosides[6].

Phytochemical Test analysis: conducted analysis for carbohydrates, lipids, phenols, terpenoids, alkaloids and other nitrogen- containing compound. Demonstrated antioxidant abilities of phytochemicals through *In vitro* and human studies.

Bio luminance test: Utilizing UV- induced fluorescence present a promising approach for achieving the bio luminance test for the investigation of plant response to the fluorescence light. This method is non- invasive and enables remote and swift recording. UV- excited green plants emit red fluorescence.

Antimicrobial Screening: The antimicrobial screening was conducted to determine the susceptibility of various bacteria to the extracted compound. This Screening involved measuring the ability of the test samples to inhibit the *In vitro* growth of bacteria using standardizing protocol.

The agar well diffusion technique was employed to evaluate the antibacterial activity of the extracted secondary metabolites. Bacterial strains, including clinically relevant pathogen s such as *Staphylococcus aureus* and *Escherichia coli* were used for the assays. The bacterial strains were sub- cultured using the streak plate technique on nutrient agar Petri plates and incubated to the mid- log phase. Fresh mid- log phase bacterial cultures were then spread on individual nutrients agar and as well as were created[8].

Antioxidant Activity Assessment: During the assessment prepared different concentration of the sample in five test tubes (e.g. 200ml, 400ml, 600ml, 800ml, 100ml) and include one test tube as a blank. Added the extraction solvent to make the volume up to 1ml. Combined 2.5ml phosphate buffer with 2.5ml Potassium Ferricyanide $K_3[Fe(CN)_6]$. Covered with aluminium foil and incubate in a water bath at 50^oC for 20 minutes.

After removing from water bath, added 2.5ml absorbance solution. Pipetted 2.5ml from each test tube and transfer it to another. Added 2.5ml distilled water followed by 0.5ml ferric chloride. Measured the absorbance at 518 to 550 nm using Nanodrop spectrophotometer and recorded the reading for both the sample and blank test tube[9].

Results and Discussion

The phytochemical analysis conducted on the extract of Wild Senna revealed the presence pharmacologically importance classes of compounds, including saponins, tannins, alkaloids, flavonoids, phenols, steroids and glycosides. However, the distribution of these compounds varied across different extracts, with certain compounds absent in specific extracts. These secondary metabolites are known for their therapeutic activities and often work synergistically or antagonistically in treating various disease (Table-1). Among these compounds, alkaloid stand out for their wide range of pharmacological activities, including antibacterial and antifungal properties. Flavonoids, on the other hand have garnered significant interest due to their potential health- promoting properties, such as antioxidant, anti-inflammatory, antiallergenic, antiviral, antiaging and anticarcinogenic activities. In addition to other water- soluble substances, these bioactive thiocyanate, nitrate, chloride, and sulphates are found naturally in the majority of plant materials and are known to have bactericidal, pesticidal, or fungicidal properties, giving plants antimicrobial properties[10]. Tannins known for their astringent and haemostatic properties, play a role in wound healing, alleviating inflamed mucus membranes and inhibiting microbial growth. The plant's antioxidant and anti-inflammatory properties are indicated by its quantitative compounds, which may also explain why *Senna obtusifolia* leaves have been used traditionally[9].

Utilizing UV- induced fluorescence present a promising approach for achieving this control, as it allows for the investigation of plant responses to the presence of oil. This method is non-invasive and enables remote and swift recording. UV- excited green plants emits red fluorescence in the presence of oil- related compounds (Figure-2).

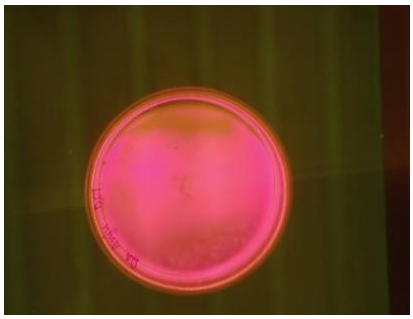


Figure-2: Test of Bioluminance confirmed that the presence of oil in sample (methanol extract).

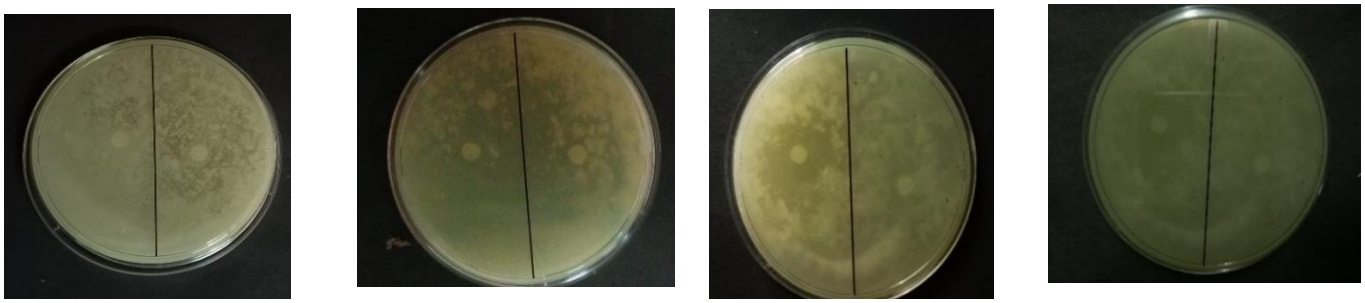
The zone off inhibition was observed around the wells containing the *Senna obtusifolia* solvent extract sample containing well as well as disc both shows antibacterial properties of Wild Senna sample with different solvents having antibacterial properties with *Escherichia coli* (Figure-3) and *Staphylococcus aureus* (Figure-4). The antimicrobial sensitivity test conducted on Wild Senna leaf extracts demonstrated solvents extraction dependent activity against test organisms.

In each plate, the solvent served as the control (left side) and the plant extract with the respective solvent was added on the well (right side). Extract exhibited varying degree of inhibition, with acetone plate extract showing the highest activity against *Staphylococcus aureus* and *Escherichia coli* no zone of inhibition was seen in *Staphylococcus aureus* plate with methanol extract.

Assessing the antioxidant activity of Wild Senna is crucial for evaluating the potential health benefits and application in various industries, including food, pharmaceuticals and cosmetics. For antioxidation assessment, Hydrogen peroxide oxygen species associated with oxidative stress. Reductio of H_2O_2 is monitored via spectrophotometry, and antioxidant activity is expressed as the percentage inhibition of H_2O_2 scavenging. Using the hydrogen peroxide scavenging assay method, the antioxidant activity of *Senna obtusifolia* was determined along with its capacity to scavenge free radicals. The colour change observed was from yellow to orange. Distilled water, acetone, methanol and ethanol were used in varying amounts (200ml, 400ml, 600ml, 800ml, 1000ml) within a specific range, with measurements taken at 520nm (Figure-5).

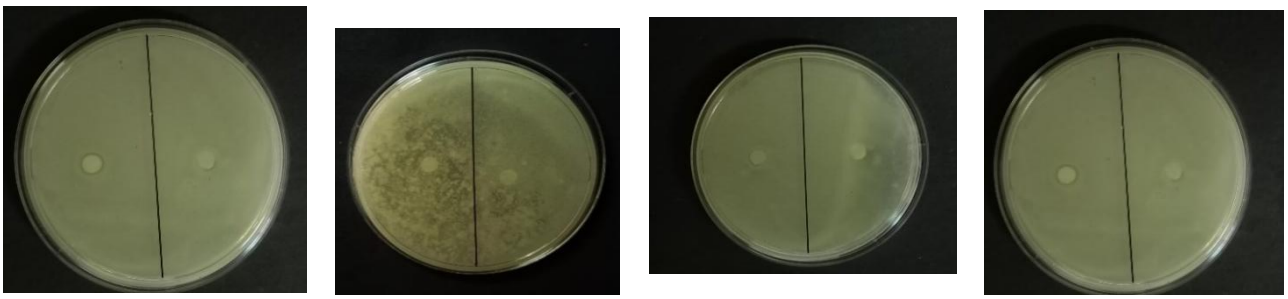
Table-1: Phytochemical analysis of Wild Senna extract using different solvent extracts. Presence of phytochemical is donated by (+) sign and absent of phytochemical is (-) sign.

Chemical constituents	Phytochemical test	Distilled water extract Sample 1	Acetone extract Sample 2	Methanol extract Sample 3	Ethanol extract Sample 4	Petroleum ether Sample 5
Alkaloids	Dragandorff's Test	+++	++	+++	++	--
	Wagner's Test	+++	++	++	+++	--
Protein	Xanthoproteic Test	++	++	++	++	+
Terpenoid Test	Salkowski Test	+++	+++	+++	++	++
Steroid Test	Salkowski Test	+++	+++	+++	++	++
Flavonoid Test	Alkaline Test	+++	--	-	--	++
Amino Acid	Ninhydrin Test	++	--	++	+	++
Tannin	Ferric chloride Test	++	-	--	--	--
Saponin	Frothing Test	++	++	--	--	--
Anthraquinone Test	Bontrager's Test	+	--	+	--	-
Cholesterol test		+++	--	++	++	



(A) (B) (C) (D)

Figure-3 Antibacterial assay with *Echerichia coli* (A) Distilled water; (B) Acetone extract; (C) methanol extract; (D) Ethanol extract.



(A) (B) (C) (D)

Figure-4: Antibacterial assay with *Staphylococcus aureus* (A) distilled water; (B) Acetone; (B) Methanol extract; (D) Ethanol extract.

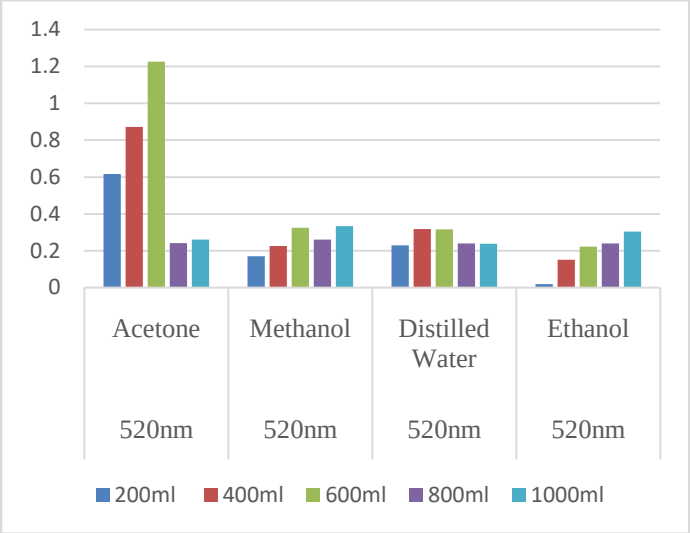


Figure-5: Antioxidation assay using Hydrogen peroxide for *Wild Senna* extract using different solvent.

These results validate the traditional folk medicinal use of Wild Senna for pain relief and the defence against microbial infections. The anti-inflammatory, anti-fungal, anti-dote, antimicrobial, and anti-yeast properties may be attributed to saponins[11]. The process of investigating these pharmacological characteristics entails using methods for extraction, purification, separation, crystallization, and identification of different bioactive substances that have therapeutic value in treating a range of human illnesses[12]. Coffee has been replaced with roasted *Senna obtusifolia* seeds. The gums from *Senna obtusifolia* seeds make a decent substitute for guar and locust bean gums[13].

Conclusion

A wide range of pharmacologically significant substances, including anthraquinone, saponin, tannin, alkaloids, flavonoids, phenols, steroids and glycosides were discovered during the thorough investigation of Wild Senna preparations. The process of identifying and extracting bioactive chemicals from Wild Senna has yielded important information on the plant's potential medicinal uses in pharmacology. The potential of Wild Senna as a source of new antimicrobial agents has been highlighted by in *vitro* studies that have revealed strong antibacterial activity of Wild Senna extracts against a broad spectrum of microorganims. Considering the therapeutic potential of medicinal plant, wild senna offers a wide range of pharmacological applications. However, to validate and understand the mechanism of action, and analyze the effectiveness and safety of Wild Senna for a range of pharmacological applications, detailed study is necessary. The phytochemical makeup and antibacterial activity of Wild Senna, therefore supports the current investigation into the discovery and development of natural product based drugs that is cost-effective and safe for the human health.

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