PHYTOCHEMICALS PROPERTIES AND ANTIBACTERIAL ACTIVITY OF COMBINED LEAVES EXTRACT OF *Senna siamea, Coffee senna and Citrus lemon*

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Abstract

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. An increasing reliance on the use of medicinal plants in societies has been traced to the extraction and development of several drugs from these plants. Standard procedure for analysis of phytocompounds that which is based on color formation or disappearance and bacterial activity was adopted. n- hexane extract shows the presence of phlobatannins, flavanoids, volatile oil, glycoside, and saponins and terpenoids also shows the absence of Alkaloid, phenols, glycoside (general), resins, and tannins while in the water extract shows the presence of Alkaloid, saponins, terpenoids, volatile oil, and the presence of tannins, glycoside, anthraquinones, resins, phlobatannins, phenols, and absence of flavanoids. In antibacterial activity (*Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, and *Streptococcus pneumonia*) shows no inhibition in the n-hexane extract while in water extracts, *Staphylococcus aureus* at 2.00 mg/dl and 1.00 mg/dl shows inhibition, *Salmonella typhi*, *E. coli*, and inactive against *Streptococcus pneumonia.* At 0.50 mg/dl it is active against *Staphylococcus aureus*, *E. coli* and inactive against *Salmonella typhi* and *Streptococcus pneumonia*. Based on our findings it can be concluded that the combined leaves extract can be used in treatment of these bacterial spacies.

Keywords: Antibacterial, Activity, Extracts**,** Leaves, Medicinal, Properties**,** Phytochemicals

1. INTRODUCTION

Health is a state of complete physical, mental, and social well being and not merely the absence of disease or infirmity Medicine [1], in several developing countries, using local traditions and beliefs, is still the mainstay of healthcare. As a basis for the maintenance of good health, most developing countries use traditional medicine and medicinal plants, which have been traced to the occurrence of natural products with medicinal properties [2].

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. An increasing reliance on the use of medicinal plants in societies has been traced to the extraction and development of several drugs from these plants [2].

Plants have in their arsenal an amazing array of thousands of chemicals noxious or toxic to bacteria, fungi, insects, herbivores, and even humans. Fortunately, this chemical diversity also includes many compounds that are beneficial to humans: vitamins, nutrients, antioxidants, anti carcinogens, and many other compounds with medicinal value. Most plant species in the world are not edible due largely to the toxins they produce which evolve from the development of a defense mechanism generated by these plants to fight off predator [3].

Plants, which have one or more of its organ containing substances that can be used for the therapeutic purpose, are called medicinal plants [4]. Medicinal plants are considered as rich resources of ingredients, which can be used in, drug development either as pharmacopoeial, non-pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and because of that, they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric [5]. Medicinal plants have some natural products, which perform definite physiological action on human body, and these bioactive substances with curative properties are perhaps due to the presence of various secondary metabolites, which includes alkaloids, carbohydrates, terpenoids, flavonoids, resins, tannin, saponins, essential oils, glycosides, phenols and steroids [6].

In some cases, the crude extract of medicinal plants may be used as medicaments, on the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important, where the active molecule cannot be synthesize economically, the product must be obtained from the cultivation of plant material. Some major plant drugs have been identified for which no synthetic one is currently available such as Vinblastine from *Catharanthusroseus* – anticancer; Quinine from *Cinchona sp*. – antimalarial; Cocaine from *Erythroxylum coca* – Topical anaesthetic; Morphine from *Papaversomniferum* – Painkiller; Codeine from *Papaversomniferum* – anticough; Atropine from *Atropa belladonna* – Spasmolytic; Cardiac glycosides from *Digitalis sp*. – congestive heart failure; Artemisinin from *Artemesiaannua* – Antimalarial, e.t.c. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance [7]. The first step in the value addition of medicinal plants bio resources is the production of herbal drug preparations (i.e. extracts), using a variety of methods from simple traditional technologies to advanced extraction techniques.

Just over 200 years ago, a 21 years old Pharmacist apprentice named Friedrich Serturner isolated the first pharmacologically active compound Morphine from opium produced by cut seeds pod of the poppy plants, Papaver Sanniferum. Drugs discovery from medicinal plants leads to the isolation of early drugs such as Cocaine, Codeine, Digitoxin and Quinine. According to [8] around 21 Natural products (NP) and NP-Derived drugs where launched on to the market in the united state, Europe or Japan from 1998 to 2004. The 21 drugs classified as $3NP_S$, 10 Semi-synthetic NP_S and 8 NP derived drugs. [9] Estimates that about 60% of the drugs that are now available including household names such as Artemisinin, Camptothecin, Lovastatin, Maytansine, Penecillin were either directly or indirectly derived from Natural Products. Approximately two-third of new drugs in the past 25 years has originated from the discovery of particular secondary metabolites derived from Natural

Biodiversity. Natural products besides being source of lead for a number of drugs also play an important role in the industrial drugs synthesis [9].

Approximately two-third of new drugs in the past 25 years has originated from a discovery of particular secondary from natural products biodiversity. Natural products, beside a source of leads for a number of drugs. The vast majority of peoples on this world still rely on their traditional Material (medicinal plants and other materials) for their everyday care needs. 80% of the world population primarily health care needs, about 25% of the drugs prescribed worldwide come from plants , 121 such active compounds being in current use, of the 252 drugs considered as basic and essential by WHO, 11% are exclusively of plants origin and significant number are synthetic drugs obtained from natural precursors, it has been estimated that as much as 37% of all pharmaceutical sales are for compounds that are derived, either wholly or impact, from natural products. *Senna siamea*, *Coffee senna* leaves are known traditionally to cure bacteria specie; hence the study was to justify part of the claim by traditional healers (herbalists).

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemicals and reagents used were of analytical grades. They were all purchased from Ps scientific ltd, Northampton UK, Guangdong chemical factory co. ltd. Shatoo China, Sigma Aldrich laboratory chemicals ltd India, Fisonplc scientific equipment division, India and Philip Haris limited Shenstone England.

2.2 Materials

Materials	Grade/Model
Conical flask	50ml, 100ml (glass)
Test tube	Glass
Measuring cylinder	Glass
Funnels	Glass
Analytical balance	Metal container/ electric Application
Sampling container	Rubber
Hot plate	Metal container/ electric Application
Oven	Metal container/ Electricity Application
Bijou bottle	Glass
Measuring ruler	Rubber
Spatula	Metal
Watch glass	Glass
Conical flask	Glass
Sterile loops	Metal
Sterile forceps	Metal
Petri dish	Glass
Pipette	Glass
Micro pipette	Glass
Incubator	Metal container and electric Application
Sensitivity filter paper disc	Paper

Chemical/ Reagents	Company
n- hexane	Ps scientific ltd, Northampton uk.
Distilled water	
Filter paper	Wattmann filter papper
Chloroform ($CHCL_3$)	Ps scientific ltd, Northampton uk.
Ethanol	Guangdong guanghua chemical factory co. ltd. Shatoo china
Ethyl acetate	Guangdong guanghuasci-Tech co., ltd china.
Ferric chloride ($Fecl_3$)	Sigma Aldrich laborchemicals ltd india.
Copper(II) sulphate	Ps scientific ltd, Northampton uk.
Glacial acetic acid	Ps scientific ltd, Northampton uk.
Ammonia (NH_3)	Philip Haris limited Shenstone Englsnd.
Carbon tetrachloride (ccl_4)	Labo chemical and laboratory reagent ltd.
Hydrochloric Acid (Hcl)	Fisonplc scientific equipment division, india.
Fehling solution A	Ps scientific ltd, Northampton uk.
Fehling solution B	Ps scientific ltd, Northampton uk.
DMSO	Ps scientific ltd, Northampton uk.

2.3 Sample collection

The plants was collected within Kashere metropolis from the available trees. The leaves were identified at the Department of Biological Sciences, Faculty of Science, Federal University of Kashere. Air-dried method was adopted in the laboratory for 6 days.

2.4 Extract preparation

25g coffee *Senna* leaves, 25g *Senna siamea* and 12g of *Citrus lemon* was weighted using weighting balance and soaked in 500ml of n-hexane respectively in a bottle for 72hours with intermittent stirring, the combined leaves were filtered using whatman No:1 filter paper and funnel, the residue of the extract were discard and a crude extract was filtered and exposed to heat so that the solvent (n-hexane) will evaporate leaving the extract in the container, the crude extract was weight using a weighting machine and a total of 12g of the crude extrac was found.

Similarly the plants was weighed at the rate of 50grams each using a weighting balance and soaked in 800ml of water respectively in a conical flask for 72 hours with stirring Whattman No:1 filter paper was used to filter the solution. The extracts were evaporated using a hot oven at 40^0C [4] the dried extract were aseptically stored in a sterile laboratory bottles until when they were used.

2.5 Phytochemicals screening

Method of [6] was used to estimate Tannins**,** Glycosides, Anthraquinone glycoside, Cardiac Glycoside, Resins, Saponins, Phlobatannins, Flavonoids, Phenols, Volatile oils and Alkaloids

2.6 Antibacterial Activity (disk diffusion method)

- The extracts were dried using oven at 45^{O}C.
- The dried extract was mixed with 5ml of DMSO to form stock extract in a bojou bottle.
- Sterile nutrient agar was prepared and allowed to cool (solidify).
- Each of the petri dish swirled carefully until the agar was spread evently allowed to cool
- After the media has been solidified a sterile corn borer(5mm) was used to bore well in each of the petri dishes
- Few drops of disk containing each concentration of the extract was dispensed in to the well bored using a sterile pipette, one plate for each organisms and concentration
- The plates were incubated at 37 ^{O}C for 24 hours

The zone of inhibition of each plate were observed and recorded by measuring the clear zone at which growths has been inhibited from the edge of the growth [10].

Preparation of McFaland turbidity standard

- Prepare 1% solution of barium chloride dehydrate (10g/L)
- Prepare 1% sulphuric Acid (10ml/L)
- Prepare the turbidity standard by pouring0.6ml of the 1% solution of barium chloride dehydrate in to a 100ml with 1% sulphuric acid.
- Fill the cylinder to 100ml with 1% sulphric acid.
- Place the turbidity standard solution in a tube identical to the one used for the broth sample.

Note: It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.

Results

Table 1: Phytochemical screening Result

S/No	Phytochemicals	n-hexane Extract	Water Extract
1	Alkaloid	-	+
2	Saponins	++	+
3	Tannins	-	++
4	Phenols	++	-
5	Phlobatannins	++	-
6	Flavanoids	++	-
7	Terpenoids	+++	+
8	Volatile oil	++	+
9	Glycoside	++	++
10	Anthraquinones	+	++
11	Resins	-	++

Key : +++ present in high amount, ++ present in moderate amount, + Present, - Absent

Table 2: Antibacterial activity of combined leaves extract of *coffee senna, sennasiamea*and *citrus limonum* leaves (zone of inhibition result)

Test organisms	Observation n-hexane Extract			Water Extract			Control
	1.00 mg/dl	0.25mg/dl	0.25 mg/dl	2.00 mg/dl	1.00 mg/dl	0.50 mg/dl	2.00 mg/dl
Staphylococcu Saureus	NA	NA	NA	12.66±0.06	12.33±0.01	12.33±0.02	10.33±0.02
Salmonella Typhi	NA	NA	NA	12.33±0.01	10.0±0.01	NA	12.00±0.20
E. Coli	NA	NA	NA	12.00±0.04	9.66±0.03	11.00±0.05	NA
Staphylococu S pneumonia	NA	NA	NA	NA	NA	NA	NA

Values are Mean ± Standard Deviation, NA: No Activity

Discussion of Result

Table 1 shows the Phytochemical screening result of n-hexane and water extract of *coffee senna*, *Senna simea* and *citrus lemon* leaves, the result of the n- hexane extract shows the presence of Phlobatannins, Flavanoids, Volatile oil, Glycoside, and Saponins, and the presence of Terpenoids in high amount, the result shows the absence of Alkaloid, phenols, Glycoside (general), Resins, and tannins.And in the case of the water extracts shows the presence of Alkaloid, Saponins, Terpenoids, volatile oil, and the presence of tannins, glycoside, Athraquinones, and resins in moderate amount, and the result reveals that Phlobatannins, phenols, and Flavanoids are absent in the water extract.

While Table 2 shows the Antibacterial Activity of n-hexane and water extract of *Coffee senna*, *Senna simea*, and *Citrus limon* leaves on the test organisms (*Staphylococcus aureus, Salmonella typhi, E. coli, and Streptococcus pneumonia*).

The result revealed that n-hexane extract of the combined leaves were inactive against the test organisms (*Staphylococcus aureus, Salmonella typhi, E. coli, and Streptococcus pneumonia*) since there is no zone of inhibition because the extracts of n-hexane are inactive against the organisms, and in the case of the water extract there is inhibition in some of the test organisms of different concentration, and different species, the result revealed that there is zone of inhibition in both of the concentrations of the test organism *Staphylococcus aureus*, the result shows zone of inhibition in the organism concentration 0.50mg/dl of 12.33±0.01, followed by concentration 2.00mg/dl with zone of inhibition of 12.66±0.01, the inhibition in 1.00mg/dl is 12.66±0.06, and the control has the least inhibition of 10.33±0.02, *Salmonella typhi*, at concentration of 2.00mg/dl it has zone of inhibition of 12.33± 0.01, and at 1.00 mg/dl it has 10.0± 0.01, at 0.50mg/dl it has no zone of inhibition, and the control has no zone of inhibition at value of 12.06±0.02. *E. coli* has the zone of inhibition 12.00±0.04 at 2.00mg/dl,

9.66±0.03 at 1.00mg/dl, 11.00±0.05 at 0.50mg/dl, and the control shows no activity, *Streptococcus pneumonia* the result revealed that there is no zone of inhibition in both of the concentration of the test organisms.

The result of the presliminary phytochemical screening of the leaves extract of coffee senna,sennasimea, and citrus lemon leaves presented in table one shows that phytochemicals are present in the n-hexane extract Phlobatannins, Flavanoids, Volatile oil, Glycoside, and Saponins, and the presence of Terpenoids in high amount, and the result shows the absence of Alkaloid, phenols, Glycoside (general), Resins, and tannins in the n-hexane extracts.

As explain in the methodology of this research most of the phytochemical result in table one were read based on the color formation or disappearance as in the caseAlkaloids, Flavanoids, glycosides, volatile oil, phenols, Tannins, Terpenes, and Terpenoids, resins, Athraquinones, formation of the color precipitate indicates their presence and in the case of Saponins the frothing formation indicates the presence of the phytocompound, the result in the study correspond to the [11], but [12] reported the absent of Alkaloid in both aqueous and alcohol extracts, although they agreed with the presence of some of Phytochemicals such as tannins, Saponins, and the absence of steroids [13] also reported the absence of steroids in *Moringa oleifera* extracts. This could be as a result of different in climatic environment at which it was planted or the physiological and its maturity at the stage of harvesting [14]

Apart from determination of nutritional value of the plants, [15] has reported the therapeutic effect of some phytochemical constituents such as tannins, cardiac glycoside against cardiovascular diseases and digestive problem. It is important factor that determined the antimicrobial properties of the leaves extracts. These result what medicinal plants antimicrobial drugs. Several authors has linked the presence of bioactive compounds to the antimicrobial properties of the plants extracts [16].

In addition Table 2 shows the result of different level of the susceptibility of the bacterial to n-hexane extracts of the combined *coffee senna*, *Senna siamea and Citrus lemon* leaves It was observed that the test organisms *(Staphylococcus aureus, Salmonella typhi, E. coli, and Streptococcus pneumonia)* were not susceptible at higher or lower concentration because there is no zone of inhibition. The extracts showed a great concentration dependent inhibition as it was observed that at concentration 0.25g of the n-hexane extract there was no inhibition. This is because the quality of the extracts constituents required to inhibits their growth was not enough at the lower concentration 0.25ml.

It could be noticed that n-hexane extract has more effective against the test organisms as compared to the water extracts. This could be because water was considered to have dipole molecules and high dielectric solvents, is not as polar as water. This means that n-hexane has a better dissolving capacity than water. This observation is in agreement with the study of [17]. They suggested that water extracts differs from other solvents because it has numerous compounds that may interact antagonistically in their overall activities. The result revealed that no activity for the water extract of 1:1 concentration but methanol extract has 9mm for both the *E.coli and Streptococcus pneumonia* and 8mm for *S. aureus* for the same 1:1 concentration, [18], [19] also discovered that Alcohol extract shows greater activity than the water extracts and implies that antimicrobial components resided more in methanol concentrations.

In the study conducted by majourie, the result shows the different extract of *M. indica* had different compounds with antibacterial activity could be due to different classes of compounds, some of the classes of compound identified in the crude extract such as Alkaloids, and Terpenoids have been reported to possess Antibacterial Activity. The study conducted by Doughari and manzara, reveals that the active components of leaves of *M. indica L.* which were extracted with cold water and organic solvents (acetone and methanol) and were tested against *Staphylococcus aureus, Staphylococcus pyogenese, Staphylococcus pneumonia, Bacillus cereus, Escherchia coli, pseudomonas typhi, and shigellaflexnerri* using the Agar well (cup plate) diffusion method. Both the acetone and methanol extracts inhibited the growth of gram positive bacteria with acetone extract exerting more activities on all the gram positive bacteria in the zone of inhibition between 15- 16mm, and a gram negative bacterium *S.typhi* (14mm) at 250mg/ml. whereas water extract was not active on any of the bacterial pathogens tested at any of the extract concentration used.

Conclusion

the Phytochemical screening result of n-hexane extract of *Coffee senna*, *Senna simea* and *Citrus limonum* leaves, the result shows the presence of Phlobatannins, Flavanoids, Volatile oil, Glycoside, and Saponins, and the presence of Terpenoids in high amount, the result shows the absence of Alkaloid, phenols, Glycoside (general), Resins, and tannins. Antibacterial Activity of leaves extract of *Coffee senna*, *Senna simea*, and *Citrus limonum* leaves on the test organisms (*Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, and *Streptococcus pneumonia*) the result revealed that n-hexane extract of the combined leaves were inactive against the test organisms (*Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, and *Streptococcus pneumonia*) since there is no activity.

Because the extract of n-hexane is inactive against the test organisms. The water extract were active against the test organisms except in the *Streptococcus pneumonia* which shows no activity.

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