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IN VITRO GROWTH INHIBITORY ACTIVITY OF SECONDARY METABOLITES FROM THE ROOTS OF *BURKEA AFRICANA* AND *COMBRETUM ADENOGONIUM* ON *SALMONELLA TYPHI*

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ABSTRACT

The roots of *Burkea africana* and *Combretum adenogonium* were separately subjected to extraction by macerating using n-hexane, chloroform, methanol and distilled water solvents in an increasing order of polarity indices. Extracts obtained in the extraction process were later screened for the presence of secondary metabolites by methods employed by Mbatchou *et al*. (2010) which revealed the presence of tannins, anthraquinones, glycosides, alkaloids, saponins, phenolics, amino acids, steroids, terpenoids and flavonoids in the root samples of both *B. africana* and *C. adenogonium*. This was an indication that roots of the two plants contained ingredients which have pharmacological effects. Of the four solvents used to extract ingredients from the roots of the two plants, methanol solvent yielded the highest weighed extracts. Methanol and distilled water extracts showed the presence of 80% and above of the tested classes of secondary metabolites. These results indicated the optimal extractable abilities of both methanol and distilled water solvents which were also evident in the antimicrobial test of the root extracts on *Salmonella typhi.* Methanol extracts of the roots of *B. africana* demonstrated growth inhibitory effects with mean values of 8.00±0.00mm, 10.33±1.33mm, 12.33±0.33mm and 13.66±0.00mm at concentrations of 500, 1,000, 1,500 and10, 000µg/ml respectively. Distilled water extracts of the same part of plant demonstrated growth inhibitory effects with mean values of 7.33±0.60mm, 9.66±0.33mm, 12.00±1.00mm and 13.00±0.33 at concentrations of 500, 1,000, 1,500 and10, 000µg/ml respectively. Methanol extracts of the roots of *C. adenogonium* demonstrated growth inhibitory effects with mean values of 5.66±0.37mm, 6.33±0.33mm, 7.33±0.32mm and 11.33±0.32mm at concentrations of 500, 1,000, 1,500 and10, 000µg/ml respectively. Distilled water extracts of the same part of plant demonstrated growth inhibitory effects with mean values of 5.00±1.00mm, 6.33±0.33mm, 8.00±0.32mm and 10.66±0.32mm at concentrations of 500, 1,000, 1,500 and10, 000 µg/ml respectively. Ciprofloxacin antibiotic drug, used as positive control in the research also demonstrated growth inhibition on the bacterial isolate. The optimal extractable abilities demonstrated by the methanol and distilled water solvents supported their use to isolate antibacterial compounds from the roots of both plants by column chromatographic method. The presence of the fore mentioned classes of secondary metabolites in the root extracts, and the growth inhibitory effects shown on *Salmonella typhi* revealed that both *B. africana* and *C. adenogonium* contained antibacterial compounds which can treat typhoid fever in humans, and can also cure other diseases which affect elephants that feed on parts of the two plants.

Keywords: Extraction, polarity indices of solvents, secondary metabolites, optimal extractable ability, ciprofloxacin antibiotic drug and growth inhibitory activity.

INTRODUCTION

The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacology. The value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007). There are about 30,000-40,000 plant species known to be in existence on earth, and climatic factors are important in giving local variations to plants in a particular habitat .Thus, soil differences are reflected in the variations of vegetation. Therefore, vegetation normally show some form of zonation (Schmidt *et al*., 2002). Tropical Africa possesses a vast array of plants which natives claimed have various curative abilities (Sofowora,1993). Ghana is blessed with a great variety of natural vegetation, some of which are used in traditional medicine to cure various sicknesses and diseases. They do not serve as source of medicinal importance only; some are also useful for ornamental purposes, while many due to odoriferous nature are useful in flavoring or as food additives and preservatives (Micheal, 1990).

The use of plants whether herbs, shrubs or trees in parts or whole in the treatment and management of diseases and

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disorders dates back to pre-historic days. According to Okanla *et al*. (1990), plant extracts have been used in folk medical practices for the treatment of ailments since antiquity. The medicinal properties for various plant materials and extracts have been recognized since the beginning of the fifth century. A rich store house of medicinal plants exists everywhere, especially in Africa which offers a vast reservoir of plants that have been categorized (Duke, 1995). World health organization (WHO) described plants as one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

The use of plant parts as medicine has served as the starting point in the development of synthetic drugs today. Through scientific methods, active ingredients have been isolated from plant parts, used as traditional medicine. Quinine was isolated from the bark of *Cinchona succirubra* and was used against the genus plasmodium which lives in the gut of *Anopheles mosquito* which spread the disease malaria (Chavez, 2002). Morphine and Codeine, also isolated from the sticky juice of oriental *Opium poppy*, were used to control severe pain and cough respectively (Egan, 2002). Vincristin, one of the most potent anti-leukemic drugs in use today, was isolated in search of diabetes treatments from *Vinca rosea* (Ernst,1999).

Presently, scientists are still in search of plants that might yield new medicinal values. Taxol, a compound isolated from the stem bark of *Taxus brevifolia* is used as anti-cancer agent (Kinghorn and Manuel, 1993). Artemicinin obtained from the leaves and flowering tops of sweet wormwood, *Artemisia annua* constitutes a class of anti malarial drugs because it is toxic to chloroquine resistant *Plasmodium falciparium* (Klayman, 1985). By molecular modification, successful anesthetics such as Benzocaine and Procaine have been obtained from cocaine, a compound isolated from *Erythroxylon coca* (Egan, 2002). It is estimated that plant materials have provided the models for 50% modern drugs. The primary benefits of using plant derived medicines are relatively safer than synthetic alternatives, offering therapeutic benefit and more affordable treatment. One can understand the important role that ethno pharmacology has played in the past. World health organization estimates that up to 80% of the world population relies on plants for their health care since western pharmaceuticals are often expensive and inaccessible. In China, for example, traditional medicine is largely based on some 5,000 plants used to treat 40% of urban patients and 90% of patient in rural areas (Shao *et al*., 1996).

In industrialized countries the use of plants has declined but has contributed to more than 7,000 different compounds in use today as laxatives, anti-cancer agents, hormones, contraceptives diuretics antibodies, decongestants analgesics, anesthetics and anti-parasitic compound (Van Wyk *et al*., 2000).The success of the health care system in most developing countries is due to the support provided by traditional medicine to that of orthodox medicine. The present health status in Ghana would not have been attained without the involvement of traditional medicine in health delivery system. Available figures show that 60% to 70% of Ghanaians rely on traditional medicine for their health needs (Boye, 1985).
The more current and most effective antibiotics are more expensive and out of reach of many Africans, majority of whom reside in rural areas. These antibiotics are also associated with some serious side effects. The cumulative effects of synthetic drugs have shifted attention to the use of herbal formulations and drugs of natural origin which could remedy the adverse effects of synthetic drugs, providing gentle nourishing actions. This has provided the ground for experimentation of a vast number of plants which have antimicrobial activities.

The purpose of this investigation is to identify solvents with optimal extractable abilities among solvents used in the research which can isolate antibacterial compounds by column chromatographic method from the roots of both *Burkea africana* and *Combretum adenogonium*. The project is geared towards identifying the possible classes of secondary metabolites present in the roots of the two plants which would be responsible for antibacterial activity. The work is also aimed at providing a scientific back-up for the use of roots of plants to treat typhoid fever.

MATERIALS AND METHODS

Materials and Sources

The root samples of *Burkea africana* and *Combretum adenogonium* were collected from Mole National Park in the Northern region of Ghana. The plants were identified by Dr. Isaac Sackey and Dr. Walters Kpikpi, both from the Department of Applied Biology, Faculty of Applied Sciences, University for Development Studies (U.D.S), Ghana.

Pure cultures of *Salmonella typhi* were obtained from Microbiology Department of Komfo Anokye Teaching Hospital, Kumasi and were sub-cultured at the Microbiology Laboratory of University for Development Studies.

Solvents such as n-hexane, chloroform, methanol and distilled water, including reagents of analytical grade were provided by Department of Applied Chemistry and Biochemistry, U.D.S.

Plant sample preparation: The root samples of the two plants were separately cut into pieces and air dried for

seven days. The dried samples were each pulverized in to fine powdered material.

METHODS

Extraction: The prepared root sample (300.0g) of *Burkea africana* was transferred into a clean Winchester bottle, and 500ml of n-hexane was added to it. The n-hexane mixture was shaken vigorously, allowed to stand for two hours and then filtered. The filtrate was concentrated using a rotary evaporator at a temperature of 40˚C to an n-hexane extract. The n-hexane residue on the filter paper obtained from n-hexane mixture was air dried overnight at room temperature, and transferred into another clean Winchester bottle. 500ml of chloroform was added to this residue to form a chloroform mixture which was subjected to the same steps as those used to arrive at the n-hexane extract and residue earlier discussed to form a chloroform extract and residue. The chloroform residue obtained was subjected to the same treatment as the n-hexane residue earlier discussed. It was then transferred into a clean Winchester bottle, and 500ml of methanol was added to it to form a methanol mixture which was subjected to the same treatment as the n-hexane mixture earlier discussed to form a methanol extract and residue. The methanol residue was subjected to the same treatment as the n-hexane residue earlier discussed. It was then transferred into a clean Winchester bottle, and 500ml of distilled water was added to form a distilled water mixture which was subjected to the same treatment as the n-hexane mixture earlier discussed to form a distilled water extract and residue.

An extraction process similar to the one employed on the prepared root sample of *Burkea africana* earlier discussed was also employed on the prepared root sample (150g) of *Combretum adenogonium* using 300ml each of n-hexane, chloroform, methanol and distilled water solvents.

The n-hexane, chloroform, methanol and distilled water extracts obtained from root samples of the two plants by extraction process were stored in distinct vials and kept at the lower compartment of a refrigerator until required for use.

Qualitative Analysis of Secondary Metabolites: Chemical tests were carried out on n-hexane, chloroform, methanol and distilled water extracts of the root samples of *Burkea africana* and *Combretum adenogonium* using standard procedures employed by Mbatchou *et al.* (2010).

Test for Tannins: A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicated the presence of tannins.

Test for Saponins: About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of saponins.

Test for Terpenoids: The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids.

Test for Steroids: Acetic anhydride (2ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids.

Test for Flavonoids: About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids.

Test for Alkaloids: The aqueous extract (3ml) was stirred with 3ml of 1%HCl on a steam bath. Mayer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids.

Test for Phenolics: About 0.2g of the extract was dissolved in 2ml of distilled water, and three drops of ferric chloride was added. A positive test resulted when the solution turned blue, green, and blue-green, and precipitated.

Test for Anthraquinones: About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose pink colour indicated the presence of anthraquinones.

Test for Glycosides: A small amount of the extract was hydrolyzed in 2ml of HCl solution, and neutralized with equal amount of sodium hydroxide solution. Few drops of Fehling's solution A and B were added. Red precipitates indicated the presence of glycosides.

Preparation of Concentrations of Extracts: Serial dilution method was used to prepare different concentrations of root extracts of *Burkea africana* and *Combretum adenogonium*, and ciprofloxacin standard antibiotic. 0.005g of each extract was dissolved in 0.5ml of dimethylsulphoxide (DMSO) in a sterile vial to obtain a stock solution of 10,000ug/ml concentration. Portions of the stock solution were diluted to obtain concentrations of 500ug/ml, 1,000ug/ml and 1,500ug/ml. Concentrations of ciprofloxacin antibiotic were also prepared in the same manner as the root extracts earlier discussed.

Preparation of Media Plates: Mueller Hinton agar was used to prepare media plates according to manufacturer's procedure.

To 19.00g of Mueller Hinton agar in a conical flask, 500ml of distilled water was added and agitated. The content was heated to boil on a hot plate with a magnetic stirrer in it until the powder completely dissolved. The content was transferred into a sterile media bottle, and autoclaved at 121^0C for 15 minutes. The medium was allowed to cool, and about 20ml was poured into each sterile Petri dish and then allowed to solidify.

Antimicrobial Sensitivity Test: The spreading method of Cruickshank *et al*., 1980 and the well agar diffusion method employed in the research work of Mbatchou *et al*., 2010 were used.

Table1. Color, texture and weight of extracts.

Plant	Extracts	Weight(g)	Texture	Colour
Burkea africana (root)	n-Hexane	0.2	Oily	Yellowish
	Chloroform	2.60	Sticky	Brownish
	Methanol	9.40	Powdery	Dark red
	Distilled water	0.60	Powdery	Dark brown
Combretum adenogonium (root)	n-Hexane	0.20	Oily	Yellowish
	Chloroform	0.40	Oily	Yellowish
	Methanol	5.60	Powdery	Dark red
	Distilled water	0.60	Powdery	Brownish

Table 2. Secondary metabolites of the root extracts of *Burkea Africana.*

Secondary metabolites	Root extracts			
	n-Hexane	Chloroform	Methanol	Distilled water
Tannins	-	-	+++	+++
Anthraquinones	-	-	++	+++
Glycosides	-	-	-	+
Alkaloids	+	+	+++	++
Saponins	-	-	+++	++
Phenolics	-	-	+++	+++
Amino acids	-	-	++	-
Steroids	+	++	+++	+++
Terpenoids	+++	+++	+++	+++
Flavonoids	+++	+++	-	-

Table 3. Secondary metabolites of the root extracts of *Combretum adenogonium.*

Secondary metabolites	Root extracts			
	n-Hexane	Chloroform	Methanol	Distilled Water
Tannins	-	-	+++	+++
Anthraquinones	-	-	+++	+++
Glycosides	-	-	+++	-
Alkaloids	-	-	+++	+++
Saponnins	-	-	+++	+++
Phenolics	-	-	+++	+++
Amino acids	-	-	+++	++
Steroids	+++	+++	+++	+++
Terpenoids	+++	+++	+++	+++
Flavonoids	+++	+++	-	-

Key: Strongly positive (+++); Moderately positive (++); Weakly positive (+); Absent (-)

A loop full of a 24 hours old culture was uniformly spread over the surface of the agar plate with a sterile bent rod. 0.1ml of the prepared root extracts and the drug at different concentrations were used to fill holes bored by 5mm borer. The plates were then incubated at 37^{o}C for 24 hours.

The diameters of the zones of growth inhibition were measured by calculating the difference between the diameter of the borer and the diameter of the zone inhibited (Mbatchou *et al.*, 2010).

RESULTS AND DISCUSSION

Table 1 results shows weight, texture and colour of root extracts of *Burkea africana* and *Combretum adenogonium.* Of the four solvents used in the extraction process, methanol solvent gave maximum weight yield of extracts. It yielded extracts with weights of 9.40g and 5.60g respectively for the two plants. Extracts obtained by the use of n-hexane solvent, were oily in texture for both *Burkea africana* and *Combretum adenogonium*. Chloroform extracts were sticky and oily respectively, while methanol and distilled water extracts were powdery. The n-hexane extracts of roots of both plants appeared yellow in colour. Chloroform extracts of plants appeared brownish for *Burkea africana*, and yellowish for *Combretum adenogonium*. A dark- red colour was observed for all methanol extracts. The distilled water extract of *Burkea africana* was dark-brown in colour, whereas the same solvent extract appeared brown for *Combretum adenogonium*. The similarities and dissimilarities in texture and colour of these root extracts can be attributed to the presence of the same or different classes of secondary metabolites in them.

In table 2 the classes of secondary metabolites present in root extracts of *Burkea africana* at varied degrees. Both the n-hexane and chloroform extracts revealed the presence of alkaloids, steroids, terpenoids and flavonoids. Steroids and flavonoids are of importance and interest in pharmacy and nutrition due to sex hormones and flavorings respectively. Tannins, anthraquinones, glycosides, saponnins and amino acids were absent in the fore mentioned extracts. The methanol and distilled water extracts, each demonstrated the presence of 80% of the tested classes of secondary metabolites. Tannins, anthraquinones, alkaloids, saponins, phenolics, steroids and terpenoids were present in these two extracts. Glycosides were present in distilled water extract but absent in methanol extract, and amino acids were present in methanol extract but absent in distilled water extract. Flavonoids were absent in both extracts.

Table 3 results represent the classes of secondary metabolites present in root extracts of *Combretum adenogonium* at varied degrees. Steroids, terpenoids, and flavonoids were present in both n-hexane and chloroform extracts, whereas tannins, anthraquinones, alkaloids, saponins, phenolics and amino acids were absent. The methanol extract revealed the presence of 90% of the tested classes of secondary metabolites. Tannins, anthraquinones, glycosides, alkaloids, saponins, phenolics, amino acids, steroids and terpenoids were present in it, while flavonoids were absent. The distilled water extract revealed the presence of 80% of the tested classes of secondary metabolites. Of the ten classes of secondary metabolites tested in this extract, only glycosides and flavonoids were absent.

Results in tables 2 and 3 showed that the methanol and distilled water solvents did not extract flavonoids from the roots of the two plants. The methanol solvent extracted amino acids from the roots of *Burkea africana,* while distilled water solvent did not. The distilled water solvent extracted glycosides from the roots of the fore mentioned plant, while methanol solvent did not. Also, the methanol solvent extracted glycosides from the roots of *Combretum adenogonium*, while distilled water solvent did not. These two solvents demonstrated optimal extractable abilities. Their extracts indicated the presence of 80% and above of the tested classes of secondary metabolites which have pharmacological effects, and account for antimicrobial properties.

From table 4 results which represent growth inhibitory activity of root extracts of *Burkea africana* and ciprofloxacin antibiotic at concentrations of 500, 1,000, 1,500, and 10,000ug/ml on *Salmonella typhi*, all tested extracts inhibited growth of the bacterial isolate in an increasing trend as their concentrations. The most toxic of the extracts on the bacterial isolate was alternating between the methanol and distilled water extracts. The methanol extract demonstrated growth inhibition on *Salmonella typhi* recording mean values of 8.00±1.00mm, 10.33± 1.33mm, 12.33±0.33mm and 13.00±0.00mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml respectively. The distilled water extract demonstrated growth inhibition on the bacterial isolate recording mean values of 7.33±0.60mm, 9.66±0.33mm, 12.00±1.00mm and 13.66±0.33mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml respectively. The least toxic of the extracts on the bacterial isolate was alternating between the n-hexane and chloroform extracts. The n-hexane extract demonstrated growth inhibition recording mean values of 5.00±0.32, 7.36±0.32mm, 8.66±0.33 and 10.00±1.00mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml, and10,000µg/ml respectively. The chloroform extract demonstrated growth inhibition recording mean values of 5.33±0.32mm, 6.66±0.33mm, 8.33±0.33mm and 9.66±0.33mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml respectively.

Table 5 results show the growth inhibitory activity of root extracts of *Combretum adenogonium* and ciprofloxacin antibiotic at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml on *Salmonella typhi*. Of the four extracts tested on the bacterial isolate, the most toxic was alternating between the methanol and distilled water extracts, whereas the least toxic was alternating between the n-hexane and chloroform extracts. The methanol extract inhibited growth of the isolate recording mean values of 5.66±0.37mm, 6.33±0.33mm, 7.33±0.32mm and 11.33±0.32mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml respectively. The distilled water extract inhibited growth of the isolate recording mean values of 5.66±0.37mm, 6.33±0.33mm, 8.00±0.32mm and 10.66±0.32mm at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml, and 10,000µ/ml respectively. The n-hexane extract at concentrations of 500µg/ml, 1,000µg/ml, 1,500µg/ml and 10,000µg/ml inhibited growth of *Salmonella typhi*, recording mean values of 3.66±0.33mm, 4.66±0.33mm, 6.00±1.00mm and7.33±0.35mm respectively. The chloroform extract demonstrated growth inhibition of the isolate, recording mean values of 4.00±0.00mm, 5.00±1.00mm, 6.00±0.00 and 8.00±0.00mm in an increasing order as the extract concentrations.

The high growth inhibitory effects demonstrated by the methanol and distilled water extracts on the bacterial isolate confirmed that methanol and distilled water solvents were optimal in the extraction process. Also, results of growth inhibition shown by the root extracts of both *Burkea africana* and *Combretum adenogonium* on *Salmonella typhi* indicated that roots of the two plants contained secondary metabolites which are antibacterial agents that can treat typhoid fever in humans, and other bacterial causing diseases which affect elephants that feed on parts of the two plants.

CONCLUSION

Methanol and distilled water solvents can be used to isolate antibacterial compounds from the roots of the two plants by column chromatographic method. The secondary metabolites present, and the growth inhibitory activity exhibited by root extracts of the two plants are indications that apart from their roots being used locally to treat *Neisseria gonorrhea* infection, they can as well be used to treat *Salmonella typhi* infection in humans, and other bacterial causing diseases which affect elephants since they also feed on parts of these plants. It is recommended that decoctions from the roots of *Burkea africana* and *Combretum adegonium* should be used to treat typhoid fever caused by *Salmonella typhi.*

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