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Preliminary Phytochemical Analysis of the Stem Bark of Frankincense Plant (*Boswellia dalzielii)*

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ABSTRACT
The study was carried out to investigate the secondary metabolites of Frankincense (Boswelia dalzielii) plant by carrying out a phytochemical screening of the ethanolic, chloroform, and aqueous extracts of the stem bark using approved standard methods for phytochemical screening. The phytochemical screening of the stem bark of the plant indicated the presence of tannins, flavonoids, steroids, and saponins in the extracts. Alkaloids were only found in the chloroform and ethanolic extracts. Glycosides were present in the ethanolic and aqueous extracts. Volatile oil was not detected in all the sample extracts. This study has scientifically justified the traditional use of Boswelia dalzielii stem bark extracts in treatment of various ailments and diseases such as ulcer, malaria, epilepsy, typhoid etc. Traditionally, a decoction of the bark is drunk as a protection against dysentery, hemorrhage, and angina. The dried and crushed bark is used in combination with other herbs to treat malaria, yellow fever, stomach ailments, and many childhood diseases (Mandal et al. 2005). It is therefore, important that the extensive phytochemicals investigation should be carried out to help in establishing the chemical profile of the frankincense (Boswellia dalzielii). It is also important that further work be conducted to investigate the cytotoxic effects of the plant in order to reveal how safe it is for medicinal use.
Key words: Phytochemicals, Metabolites, Frankincense, Flavonoids, Alkaloids.

INTRODUCTION
Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since time immemorial. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadian civilizations in about the third millennium BC. One of the ancient authors, who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Medicinal plants have always been considered as a source for healthy life for people. Therapeutic properties of medicinal plants are very useful in healing various diseases and the advantages of these medicinal plants are natural (Kalemba and Kunicka, 2003). Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient's plant chemical compounds or bioactive components are often referred to as phytochemicals

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(phyto in Greek translation meaning 'plant') or phyto constituents are responsible for protecting the plant against microbial infections or infestations by pests (Doughari *et al.*, 2009). The study of natural products on the other hand is called phytochemistry. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources (Doughari, 2009). The science of application of these indigenous or local medicinal remedies including plants for treatment of diseases is currently called ethno pharmacology but the practice dates back since antiquity. A plant in which one or more of its organ contains substances that can be used for therapeutic purposes on which are precursors for the synthesis of useful drugs are called medicinal plants. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have preventive and curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants and are useful for humanity (Okigbo, 2000). In view of many diseases defiling drugs, health practices are now changing from curative to preventive medicine. Phytochemicals popular in preventive medicine are flavonoids, polyphenols, saponins, lignoids and vitamins. Also, a knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, which are precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Kayode, 2008). This study was carried out to investigate the secondary metabolites of Frankincense plant *(Boswelia dalzielii)*.

MATERIALS AND METHODS

Materials, equipment and apparatus for the study. The following glass wares and apparatus were used in the course of the work. Beaker (1000 mL, 500 mL, 250 mL, 100 mL, and 50 mL), funnel, glass rod, test tube, spatula, mortar, pestle, measuring cylinder (100 mL and 250 mL), filter paper, conical flash (250 mL), dropping pipette, water bath, weighing balance, test tube rack, test tube brush, retort stand, climb, test holder and separating funnel.

Sample and SAMPLING techniques

The frankincense (*Boswellia dalzielii*) was systematically identified, confirmed and authenticated by the Department of Biology, College of Education Zing, Taraba state, Nigeria. A tree was sampled out, and the stem bark was collected.

Sample Preparation

The plant stem bark was air dried under room temperature and pulverized (using mortar and pestle) into fine power which was sieved with a mesh and kept in an air trial container for the analysis.

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Reagents/Chemicals

All the reagents that are used in this work were of analytical; Ethanol (BDH chemicals Ltd Poole England), Hydrochloric acid, Mayer's reagent, Fehling's solution, Iron (III) Chloride solution, Sodium hydroxide, Wagners reagent.

Preparation of Chemical/Reagent

1. Wagner's reagent: 2g of Iodine and 6g of KI were dissolved in 100 mL of distilled water.
2. Mayer's reagent: 1.3g of HCl and 5g KI dissolved in 100 mL of distilled water
3. Aqueous HCl (1.5%): 1.5 mL of concentrated HCl was dissolved in 100ml of distilled water
4. Aqueous $FeCl_3$ (5%): 5g $FeCl_3$ was dissolved in 100 mL of distilled water.
5. Aqueous NaOH (10%): 10g of NaOH pellet was dissolved in 100 mL of distilled water.
6. Fehling Solution A: 35g of hydrated $CuSO_4$ dissolved in 100 mL of water and few drops of concentrated H_2SO_4 was added and diluted to 500 mL with water. Fehling Solution B: 60g of pure NaOH and 173g of Rochette salt [sodium potassium tertarat] were dissolved in 500 mL of water and filtered.

Extraction/Fractionation.

50g of the plant material was macerated with 250 mL of aqueous ethanol to obtain the crude extract. The crude extract obtained was filtered, air dried and kept at room temperature. The crude extract was fractionated by dissolving 10g of it in a mixture of 100 mL of water and 50 mL of chloroform in separation funnel, the mixture was shaken for 10 minutes and allowed to separate into two distinct layers; aqueous and chloroform. The fractions were collected into separately weighed and labeled beakers. The aqueous and chloroform fraction was subjected to phytochemicals screening.

Phytochemicals Screening

The crude extract, aqueous and chloroform fractions were subjected to the following standard methods of phytochemical screening (Harborne, 1998; Abo et al., 1999; Kindo et al., 2016) to reveal the presence or absence of the respective classes of metabolites.

Test for Flavonoids

To 3 mL of the test extract, 2 mL of 10% NaOH was added in a test tube. Appearance of yellow coloration was observed which indicates the presence of flavonoids.

Test for Tannins

2-3 drops of 5% of $FeCl_3$ solution was added to 3 mL of the extract in a test tube. A black coloration indicates the presence of tannins.

Test of Alkaloids

2 mL of the test extract was added to 2 mL of 10% aqueous HCl and was treated with a few drops of Wagner's reagent. 1 mL of Mayer's reagent was added, turbidity to precipitation was observed with all of these reagents which suggest the presence of alkaloids.

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Test for Glycosides

To 5 mL of the test extract in a test tube, 2.5 mL of H_2SO_4 was added. The mixture was heated to boiling water for 15 minutes, then cooled and neutralized with 10% of NaOH. 5ml of Fehling solution was added and the mixture boiled. A brick-red precipitate indicates the presence of glycosides.

Test for Steroids

3 mL of chroloform was dissolved in 0.5g of the test extract in a dry test tube. To the solution, 2 mL of H_2SO_4 was carefully added to form two distinct layers. A reddish-brown coloration was observed at the interface which indicates the presence of steroids.

Test for Volatile Oils

To 2 mL solution of the test extract was added 0.1 mL of dilute NaOH and a small amount of dilute HCl. The presence of white precipitate indicates volatile oils.

Test for Saponins

To 2.5 mL of the test extract was added 2.5 mL of the Fehling solutions A and B. A blue-green precipitate shows the presence of Saponins.

RESULT AND DISCUSSION

The result of the phytochemical screening of the crude extract, chloroform and aqueous fractions of the stem bark of frankincense are presented in Table 1. All the fractions showed the presence of flavonoids, tannins, steroids, and saponins in varying concentrations as highlighted on the table. However the presence of volatile oils was not detected in all the extracts. Alkaloids were not present in the aqueous fraction of the extract. Glycosides also were not present in the chlorofoam fraction of the extract. Table 1.2 Phytochemical screening of the Crude Extract, Aqueous and Chloroform Fractions of the Frankincense Stem Bark.

Metabolites	Crude Extract	Chloroform Fraction	Aqueous Fraction
Flavonoids	+	++	+
Tannins	+++	+	++
Alkaloids	++	+++	-
Glycosides	+	-	+
Steroids	+++	+++	+++
Volatile oil	-	-	-
Saponins	+++	+++	+++

KEY: +++: present in large amount, ++: present in moderate amount, +: present in trace amount, - : not detected.

It can be deduced from the result shown in the table above that the fractions in which the metabolites are found in large amount enhanced their extraction as compared with the ones in which they are present in trace amount. Edeoga et al., (2005) reported that alkaloids, tannins, saponins and flavonoids were known to show medicinal activity as well as exhibiting physiological activity. They also show anti-oxidant, anti-inflammatory and

membrane stabilizing property (Perenz et al., 1995). Kam and Liew (2002) reported that alkaloids are known to be the largest group of secondary metabolites in plants. Their presence in significant amount is claimed to have powerful effects on humans and hence could be used as pain killer medications, anti-malaria, anti-microbial, stimulants, anti-diabetic, anti-cancerous, and anti-oxidants (Duke and Ayensu, 1985; Yahaya et al., 2012). Flavonoids have inherent ability to modify the body reactions to allergens, virus and carcinogens. They show anti allergic, anti-microbial and anti-cancer activity by which it can be used for different diseases that are generally found in human body. They also help to strengthen capillary walls (Rievere et al., 2009). The presence of tannin is also important, as it forms irreversible complexes with prolin-rich protein, which results in protein synthesis inhibition (Shimada et al., 2006). Parekh et al. (2007) also reported that tannins react with proteins to provide tanning effect that helps in the treatment of inflamed ulcerated tissues. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and useful in the treatment of intestinal disorders like diarrhea and dysentery, wounds, sprains, bruises and arresting bleeding (Oghenejobo et al., 2014; Yahaya et al., 2012). Tanins are known to inhibit tumor growth, and hence could be used for cancer prevention. Thus it can be suggested from the above that *Boswellia dalzielii* is a source of bioactive compound that could have effect on the treatment and prevention of cancer.

Tannins also have potential values such as cytotoxic and anti neoplastic properties (Aguinaldo et al., 2005). Saponins from plants have long been employed for their detergent properties. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. They are used medically in hypercholesterolaemia, hyperglycaemia, antioxidant, anticancer, anti- inflammatory and weight loss etc, (Ngbede et al., 2008). Seigler (1998) reported that saponnins have anticarcinogens' properties, immune modulatory activity and cholesterol lowering activity. It is also been reported to have anti-fungal properties (Sodipo et al., 1991). Some saponins glycosides are cardiotonics while others are contraceptives and precursors for other sex hormones (Evans 2002). Plants steroids have cardiotonic activity, possess insecticidal and anti-microbial properties. It is generally used in herbal medicines and cosmetics. Glycosides are molecules in which a sugar is bound to a non-carbohydrates moiety, usually a small organic molecule. Glycoside can suppress and soothe the irritants dry coughs. They have helpful sedative and relaxant effects on the heart and muscles when taken in small doses and are diuretic (Nwinyi et al., 2004).

CONCLUSION

The results of this study clearly indicate that the preliminary phytochemical analysis of *Boswellia dalzielii* revealed the presence of flavonoids, tannins, steroids, saponins, alkaloids and glycosides which are compounds capable of causing varied physiochemical and pharmacological effects while volatile oils were absent. The diversity of phytochemicals present suggests that the stem bark of frankincense plant could be used in the development of new pharmaceuticals that address unmet therapeutic use. Furthermore, isolation, purification and characterization of the phytochemicals found present will make interesting studies.

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