**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF**

**EXTRACT FROM THE LEAVES OF *ADANSONIA DIGITATA***

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**A PROJECT REPORT SUBMITTED TO FACULTY OF PHYSICAL SCIENCE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, BAYERO UNIVERSITY KANO (B.U.K), IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) INDUSTRIAL CHEMISTRY**

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**CERTIFICATION**

This is to certify that this project was undertaking by **ABDULHAKEEM ABDULLATEEF PSC/17/IDC/00013** and been read and approved by the undersigned as meeting the requirement for the award of Bachelor of Science in Industrial Chemistry by the Department of Pure and Industrial Chemistry, Bayero University Kano.

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**DEDICATION**

I dedicate this project report to Almighty Allah (Subhanahu Wa Ta'ala) The Most Beneficent The Most Merciful Who made it possible for me to carry out this research successfully, and to my Parents, Siblings and Friends for their encouragement, support and prayers.

**ACKNOWLEDGEMENT**

In the name of Allah The Most Beneficent The Most Merciful, I Praise Allah (Subhanahu Wa Ta'ala) and Send Blessings and Salutations upon The Prophet Muhammad (Sallallahu Alaihi Wa Salam), Members of His house hold, Companions and the entire Muslim ummah, May Almighty Allah Bless them all.

All thanks be to Almighty Allah Who has spared my life, guide me and provide for me this great opportunity to successfully complete this academic research work.

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**ABSTRACT**

*Adansonia digitata* (Malvaceae) is commonly known as baobab tree native to Africa. Baobab is a multi-purpose tree which offers protection and provides food, clothing and medicine as well as raw material for many useful items. The plant leaves were collected from Dakata Kawaji, Nasarawa Local Government Area of Kano State. The leaves of the plant were air-dried under shade at room temperature with good ventilation and ground into powder. 150g of the powdered plant was then percolated with Ethanol (600ml) for two weeks. The extract was macerated using n-hexnane, Chloroform, Ethyl acetate and Methanol. The crude extract was labeled F1aa, and the fractions were labeled F2aa, F3aa, F4aa, and F5aa respectively. The results of Phytochemical screening revealed the presence of Saponins, Flavonoids, Sterols, Tannins, Alkaloids and Tri-terpenoids in some of the test fractions. The ethyl acetate fraction contains most of the compounds (flavonoids, sterols, tannis and alkaloid). The Antimicrobial sensitivity of the leaves extract and fractions were evaluated using agar well diffusion method with standard controls of Ciprofloxacin and Ketoconazole against Bacterial and Fungal isolates respectively. The prepared concentration for the fractions are 4000ug/ml, 2000ug/ml, 1000ug/ml and 500ug/ml. All the fractions were found to be sensitive at different concentration. The results of the sensivity test shows that the chloroform extract of the plant exhibited a higher zone of inhibition of 16mm and 13mm against *S. aureus* and *A. niger* at a concentration of 4000ug/ml respectively, the ethyl acetate extract of the plant exhibited a higher zone of inhibition of 16mm against *E. coli* at a concentration of 4000ug/ml, while the n-hexane extract exhibited a higher zone of inhibition of 15mm against *C. albican.*

**CHAPTER ONE**

**1.0 INTRODUCTION**

**1.1 Background of Study**

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. As there are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine (Chitme *et al.,* 2003). About 80% of the people in developing countries use traditional medicines for their health care. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens (Coman, 1999).

**1.2 Review on *Adansonia digitata***

*Adansonia* *digitata* and its related species belong to the family of Malvaceae. The tree is of African origin known for its medicinal and nutritional value. It has excellent antioxidant and anti-inflammatory properties, various parts of the tree are used to treat different types of ailments (Kamatou *et al.,* 2011). *Adansonia digitata* commonly called African baobab is a very long-lived tree, it is said that some trees are over 1000 years old. Earlier attempts to describe African Baobab on the basis of fruit difference are not accepted till now as they are not grown agronomically or domesticatedly (Chevalier, 1906). *Adansonia digitata* (Malvaceae) is commonly found in the thorn woodlands of African savannahs, which tend to be at low altitudes with 4-10 dry months per year. It tends to grow as solitary individuals, though it can be found in small groups depending on the soil type. It is not found in areas where sand is deep. It is sensitive to water logging and frost. All locations where the tree is found are arid or semi-arid (Salim *et al.,* 2012). *Adansonia digitata* (Malvaceae) is a majestic tree revered in Africa for its medicinal and nutritional value. The trees can tolerate high temperatures and long spans of drought, and are grown for their sour fruit and leaves. The plant parts are used to treat various ailments such as diarrhea, malaria and microbial infections (Kamatou *et al*., 2011).

**1.2.1 Taxonomic description and habitat**

Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Malvales; Family: Malvaceae; Genus: *Adansonia*; Species: *digitata*; Botanical name: *Adansonia digitata*; English name: Baobab (Bosch *et al.,* 2004). *A. digitata* is a massive deciduous tree, up to 20-30m tall with a diameter up to 2-10m at adult age. The trunk is often of vast girth. The bark is smooth, reddish brown to grey, soft and possesses longitudinal fibers*. A. digitata* is highly branched. The tree produces an extensive lateral root system until 50m from the trunk. The roots tips are often in the form of tubers. But the main roots of old trees are relatively shallow and rarely extend beyond 2m depth. Therefore they are very sensitive to strong winds and can be uprooted by storm (Sidibe and Williams, 2002).

**1.2.2 Leaves of *Adansonia digitata***

The leaves of Baobab tree are a staple for many populations in Africa, especially the central region of the continent. The Leaves are alternate at the ends of branches or occur on short spurs on the trunk. They are typically sun-dried and either stored as whole leaved or pounded and sieved into a fine powder (Gebauer *et al.,* 2002). Recent phytochemical analysis of the leaves of *Adansonia* *digitata* revealed that they contain a rich amount of reducing sugars, flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins, phenols, and cardiac-active glycosides (Abiona *et* *al., 2015).* Besides, the leaves have an abundant amount of mucilage, carbohydrate (60-70%), protein (13-15%), fiber (11%), fat (4-10%), and minerals including calcium, iron, potassium, magnesium, phosphorous, zinc, and manganese (Namratha and Baobab, 2015). Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (Sidibe *et al.,* 2002).

**1.3 Natural Antimicrobial Agent**

Chemical compounds having pharmacological and biological activity and produced by living organisms are called natural products. Living organisms produce primary and secondary metabolites (Krug *et* *al*., 2008). Primary metabolites are the products that have essential function in the organism, while secondary metabolites could simply be waste products or could have some important function in their producers. Secondary metabolites possessing antimicrobial activity are called the natural antimicrobials and could be extracted from different sources like plants (fruits, vegetables, seeds, herb, and spices), animals (eggs, milk, and tissues), and microorganisms (fungi and bacteria) (Medema and Blin, 2011). With special reference to plants, secondary metabolites are found to be healthy ingredients that work as antimicrobials or disease-controlling agents (Atarés *et al.,* 2010). Owing to the potential of antimicrobials against pathogenic and spoilage microorganisms, these secondary metabolites gain much importance for the application in food products (Wyatt *et al.,* 2013). Antimicrobial components of plant origin include flavonoids, thiosulfinates, glucosinolates, phenolics, organic acids, flavonoids, and saponins (Negi *et al.,* 2005). However, the main compounds with antimicrobial activity are phenols which include terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids (Cicerale *et al*., 2012).

**1.3.1 Antimicrobial Agent and Food Safety**

Traditional food preservation methods are less efficient in reducing the growth of food-borne pathogens in food products, and the ever-increasing demand for chemical-free food has paved the way for antimicrobials to be used in food industry (Jagtap *et al.,* 2009). The use of antimicrobials is a new technology by the food industry to increase the shelf life of food and overcome the issues of food quality and safety. These antimicrobials could be of natural or synthetic type, but natural antimicrobials are gaining much importance than synthetic ones. Even though synthetic preservatives are approved by government agencies for human use, many of these preservatives still threaten our health. Thus, researchers give more importance toward the potential of natural products for their antimicrobial activities (Elgayyar *et al*., 2001).

**1.4 Aim and Objectives**

**1.4.1 Aim**

The aim of these research work is to evaluate the Phytochemicals present and Antimicrobial activity of the leave extract of *Adansonia* *Digitata* (Baobab). The phytochemical screening is to investigate the presence of secondary metabolites (Saponins, Flavonoids, Sterols, Tannins, Alkaloids, Tri-terpenoids) in the leave extract which have been macerated using various solvent (n-Hexane, Chloroform, Ethyl acetate, Methanol). And also to evaluate the Antimicrobial activity of the leave extract with standard controls of Ciprofloxacin and Ketoconazole against Bacterial and Fungal isolates respectively.

**1.4.2 Objectives**

1. To collect the leaves of *Adansonia digitata*, air dry under shade and ground into powder.
2. To percolate the powdered leaves with Ethanol for two weeks.
3. To carry-out maceration with various organic solvent in polarity gradient.
4. To carry-out phytochemical screening of various fractions of the leave extract.
5. To carry-out the antimicrobial activity of various fractions of the leave extract against Bacterial and Fungal isolates.

**CHAPTER TWO**

**2.0 MATERIALS AND METHODS**

**Plant Co****llection**

**Drying, Grindin****g and seiving**

**Percolation with eth****anol (for two weeks)**

**Extraction of crude extr****act**

**n-Hexane insoluble fraction.** **Maceration with n-Hexane**

**Maceration with Chloroform**  **Chloroform insoluble fraction**

**Ethyl acetate insoluble fraction.** **Maceration with Ethyl acetate**

**Maceration with Methanol**

**Scheme 1: Fractionation process of the crude extract**

**2.1 APPARATUS EQUIPMENT AND REAGENTS**

|  |  |
| --- | --- |
| **APPARATUS AND EQUIPMENT** | **REAGENTS** |
| Beaker  Conical flask  Weighing Balance  Measuring Cylinder  Funnels  Filter Paper  Micro Syringe  Spatula  Aluminium foil  Masking Tape  Test Tube  Swab Stick  Cork Borer  Petri Dish  Bijour Bottle | Ethanol  Methanol  n-Hexane  Concentrated HCl  Distilled Water  Concentrated Sulphuric Acid  Dimethyl Sulphur Oxide (DMSO)  Ferric Chloride  Magnesium Powder  Acetic Anhydride  Mayer Reagent  Wagner Reagent  Chloroform  Nutrient Agar ( NA and PDA)  Normal Saline |

**2.2 METHODOLOGY**

**2.2.1 General Procedure**

All solvent and chemical used in this research work were of high grade. All the glass wares used were thoroughly washed using detergent and water and then rinsed with distilled water and appropriate solvent.

**2.2.2 Plant Collection**

The leaves of *Adansonia digitata* were collected from Dakata Kawaji, Nasarawa Local Government Area of Kano State. The leaves of the plant were washed, air-dried under shade at room temperature with good ventilation and ground into powder using mortar and pestle.

**2.2.3 Mode of Extraction**

The sieved powder of the plant (150g) was percolated with Ethanol (600ml) for two weeks. The extract was decanted and filtered. The solvent was allowed to evaporate at room temperature, weighed and labeled as **F1aa** (10g).

**2.2.4 Maceration of the Crude Extract**

The crude extract was divided into two and one of the half was macerated with n-Hexane, Chloroform, Ethyl acetate and Methanol.

**2.2.4.1 Maceration with n-Hexane**

Starting with n-Hexane which is the least in polarity, the crude extract (5g) was macerated wit n-Hexane (50cm3), the n-hexane soluble fraction was allowed to evaporate at room temperature and labeled as **F2aa** . The insoluble residue recovered was subjected to the next step.

**2.2.4.2 Maceration with Chloroform**

The insoluble residue of n-hexane was macerated wit chloroform (50cm3), the chloroform soluble fraction was allowed to evaporate at room temperature and labeled as **F3aa**. The insoluble residue recovered was subjected to the next step.

**2.2.4.3 Maceration with Ethyl Acetate**

The insoluble residue of chloroform was macerated wit ethyl acetate (50cm3), the ethyl acetate soluble fraction was allowed to evaporate at room temperature and labeled as **F4aa**. The insoluble residue recovered was subjected to the next step.

**2.2.4.4 Maceration with Methanol**

The insoluble residue of ethyl acetate was macerated with methanol (50cm3), the methanol soluble fraction was allowed to evaporate at room temperature and labeled as **F5aa**.

**2.2.5 Preparation of Reagent**

1. **5% Ferric Chloride solution**

In a volumetric flask 5.0g of ferric chloride was added to 100ml of distilled water. The mixture was stirred until the solution homogenized.

1. **Mayer Reagent**

1.35g of mercury chloride was dissolved in 50ml of distilled water. 5g of potassium iodide was also dissolved in 50ml distilled water. The two solutions obtained were mixed together and a 100ml Mayer’s reagent was obtained.

1. **1% HCl Solution**

1ml of concentrated HCl was dissolved in 2.80ml of distilled water and 1% HCl solution was obtained.

1. **Wagner Reagent**

2g of iodine and 6g of potassium iodide was dissolved in 100mL of distilled water and a Wagner reagent was obtained.

**2.2.6 Phytochemical Screening**

The various fractions ( **F1aa, F2aa, F3aa, F4aa,** and **F5aa )** were screened for phytochemical such as Saponins, Flavonoids, Sterols, Tannins, Alkaloids and Tri-terpenoids.

**2.2.6.1 Test for Saponins**

The test fractions (2ml) in a test tube was vigorously shaken with distilled water and allowed to stand for a while. A persistent frothing indicate the presence of Saponins.

**2.2.6.2 Test for Flavonoids**

A little amount of magnesium Powder and few drops of concentrated HCl were added to the test fractions (2ml) in a test tube. An intense red colouration indicates the presence of Flavonoids.

**2.2.6.3 Test for Sterols (Salkowski test)**

Concentrated Sulphuric acid (2ml) was added to the test fractions (2ml). A red coloration indicates the presence of Sterols.

**2.2.6.4 Test for Tannins**

5% ferric chloride drops were added to the test fractions (2ml) in a test tube. A dirty green precipitate indicates the presence of Tannins.

**2.2.6.5 Test for Alkaloids**

1% HCl (2ml) was added to the test fractions (2ml) in a test tube. The solution was treated with few drops of Mayer’s and Wagner reagent respectively. A creamy white that is Mayer and a reddish brown Wagner indicates the presence of Alkaloids.

**2.2.6.7 Test for Tri-terpenoids**

To 2ml of the test fractions chloroform (2ml) was added on addition of few drops of concentrated Sulphuric acid, a reddish brown ring formation indicates the presence of Tri-terpenoids.

**2.2.7 Antimicrobial Activity**

**2.2.7.1 Sensitivity disc preparation**

Sensitivity dics were punched from whatman No. 1 filter paper and then sterilized in bijou bottles by autoclaving at 121oC for 15minutes. The stock solution for the various fractions was prepared using Dimethyl Sulphur Oxide (DMSO). 8mg of the test fractions were dissolved in 1ml DMSO to obtain 8000ug/ml which serve as the stock solution. From the stock solution four concentrations were prepared by half serial dilution. The stock solution was divided into two half 0.5ml each. One of the half serve as the 4000ug/ml Concentration, 0.5ml DMSO was then added to the other half to make up to 1ml. The solution was divided also into two half 0.5ml each, one of the half serve as the 2000ug/ml Concentration,0.5ml DMSO was then added to the other half to make up to 1ml. The solution was divided also into two half 0.5ml each, one of the half serve as the 1000ug/ml Concentration. 0.5ml DMSO was then added to the other half to make up to 1ml. The solution was divided also into two half 0.5ml each, 0ne of the half serve as the 500ug/ml Concentration.

**2.2.7.2 Test Organism**

The test organisms that were used in this research are bacterial and fungal isolates namely *Staphylococcus aureus, E. coli, Aspergillus niger* and *Candida albican.* Respiratory tract of the isolates were collected from the microbiology laboratory of Aminu Kano Teaching Hospital (AKTH) Bayero University Kano, Nigeria and maintained on nutrient agar slants in the refrigerator (4oC) prior to use. Ciprofloxacin at 500ug/ml and Ketoconazole at 200ug/ml were the selected antibiotics used for the control against the test organisms Bacteria and Fungi respectively.

**2.2.7.3 Inoculum Standardization**

A loopful of the test isolates was picked using sterile wire loop and emulsified in 3 to 4mls of sterile physiological Saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Cheesbrough, 2000).

**2.2.7.4 Antimicrobial Activity Test**

The sensitivity of each test organisms to the various extracts were determined using well agar diffusion technique. The standard cultured organism were swabbed all over the surface of the sterile nutrient agar in petri-dish using a swab stick. Using a sterile cork borer 6mm diameter, the well were made on the nutrient agar plate. Into each well 0.1ml of the graded concentration were put into the well including the control. The plate were inverted and allow to stand for 30 minute for the extract to diffuse into the agar after which the plate were incubated aerobically at 35oC for 18 hours. This was then followed by measurement of zone of inhibition formed by the test organisms around each extract and standard antibiotic disc.

**CHAPTER THREE**

**3.0 RESULT AND DISCUSSION**

**3.1 Result**

The powdered plant (150g) yield 10g of ethanolic extract and 5g was used for maceration with n-Hexane, Chloroform, Ethyl acetate and Methanol, so also the weight, nature and color were observed as shown in table 3.1 below.

**Table 3.1: Physical Properties of Various Fractions Obtained**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Extracts** | **Symbol** | **Weight (g)** | **Nature** | **Color** |
| Ethanol  n-Hexane  Chloroform  Ethyl Acetate  Methanol | **F1aa**  **F2aa**  **F3aa**  **F4aa**  **F5aa** | 10  3.1  2.4  1.8  2.3 | Hard and glossy  Oily  Semi-oily  Gummy  Hard and glossy | Dark green  Green  Dark green  Dark green  Dark brown |

**3.1.1 Result of Phytochemical Screening**

The result of Phytochemical screening shows the presence of Saponins, Flavonoids, Sterols, Tannins, Alkaloids and Tri-terpenoids in some of the test fractions. Those that are absent are also shown in table 3.1.1 below.

**Table 3.1.1: Result of Phytochemical Screening**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Extracts** | **Saponins** | **Flavonoids** | **Sterols** | **Tannins** | **Alkaloids** | **Tri-tepenoids** |
| **Ethanol**  **n-Hexane**  **Chloroform**  **Ethyl Acetate**  **Methanol** | **+**  **+**  **+**  **-**  **-** | **-**  **-**  **+**  **+**  **+** | **+**  **-**  **-**  **+**  **+** | **-**  **+**  **-**  **+**  **-** | **-**  **-**  **+**  **+**  **-** | **+**  **+**  **-**  **-**  **+** |

**Keys: + = Present and - = Absent**

**3.1.2 Result of Antimicrobial Activity of *Adansonia Digitata***

The result ofantimicrobialactivity of *Adansonia Digitata* extract in concentration of 4000ug/ml, 2000ug/ml, 1000ug/ml and 500ug/ml against *Staphylococcus aureus, E. Coli, Aspergillus niger* and *candida albican* measured in mm of the zone of inhibition are shown in table 3.1.2 below.

**Table 3.1.2: Result of Antimicrobial Activity**

|  |  |  |
| --- | --- | --- |
| **Extracts** | **Concentration (ug/ml)** | **Diameter Zone of inhibition (mm)**  ***S. aureus. E. coli. A. niger. C. albican*** |
| **Ethanol**  **n-Hexane**  **Chloroform**  **Ethyl Acetate**  **Methanol**  **Ciproflaxacin**  **Ketoconazole** | **4000**  **2000**  **1000**  **500**  **4000**  **2000**  **1000**  **500**  **4000**  **2000**  **1000**  **500**  **4000**  **2000**  **1000**  **500**  **4000**  **2000**  **1000**  **500**  **500**  **200** | **14 11 13 12**  **10 9 10 8**  **9 7 8 00**  **00 00 7 00**  **14 13 12 15**  **10 11 8 12**  **8 9 00 10**  **00 7 00 8**  **16 15 13 14**  **13 12 9 10**  **10 9 7 8**  **00 8 00 7**  **12 16 11 13**  **10 14 9 10**  **7 12 7 8**  **00 10 00 7**  **15 12 11 13**  **13 10 8 11**  **11 00 7 9**  **9 00 00 7**  **25 30 \_ \_**  **\_ \_ 19 21** |

**Keys: mm=millimeter unit used for measuring**

**ug=microgram unit used for concentration of plants extracts**

**3.2 DISCUSSION**

In table 3.1 ethanol is the solvent with high yield of the plant extract (10g) after the extraction. In addition, the extracts also differ in terms of coloration and nature.

In table 3.1.1 the result of Phytochemical screening shows the presence of Saponins, Flavonoids,Sterols, Tannins, Alkaloids and Tri-terpenoids in some of the test fractions. The ethyl acetate fraction contains most of the secondary metabolites (flavonoids, sterols, tannis and alkaloid). Phytochemical screening of plants is significant because it helps identify new sources of therapeutically important compounds and may lead to drug discovery and development. Many phytochemical constituents found in plants are known to be responsible for anti-inflammatory, antioxidant, antilarvicidal, and antimicrobial activities. Phenolics, alkaloids, and some flavonoids are free radical scavengers that can contribute to the suppression of oxidative stress and anti-inflammatory effects in the human body that play significant roles in the treatment of various diseases (P. Tiwari et al., 2011).

In table 3.1.2 The results of the sensivity test shows that the chloroform extract of the plant exhibited a higher zone of inhibition of 16mm and 13mm against S. aureus and A. niger at a concentration of 4000ug/ml respectively, the ethyl acetate extract exhibited a higher zone of inhibition of 16mm against E. coli at a concentration of 4000ug/ml, while the n-hexane extract exhibited a higher zone of inhibition of 15mm against C. albican.The antibacterial activity exhibited by the fractions may be related to the presence of saponins, tannins in addition to flavonoids that are reported to be responsible for antimicrobial properties of some ethnomedicinal plants (Bukar et al., 2010). In support of these current findings, chloroform extract, ethyl acetate extract and n-hexane extract showed greater inhibitory activity, this may be due to better solubility of the active components in ethanol.

**3.3 CONCLUSION**

The research shows that leaves extract of *Adansonia Digitata*  have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for food safety, medications and pharmaceuticals.

**3.4 RECOMMENDATION**

Individuals, societies, socio-groups, and governmental and non-governmental organizations should devise plans which could assist in the conservation of these medicinal plants in order to prevent their extermination and exploitation of indigenous populations, as well as considerations for cultural disruptions should one or more of these plant species become a valuable resource. The antimicrobial activities of several compounds isolated from Medicinal Plant have provided interesting leads which require further investigation. It is vital that the efficacy and safety of traditional medicines be validated and their active constituents be identified so that reliable quality controls can be established.

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