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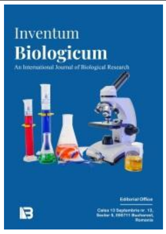

Research paper

Phyto-Chemical Investigation of Selected Medicinal Plant Species

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ABSTRACT

A significant public health issue is the development and spread of resistant microorganisms. There is a dire need for effective therapeutic options, especially those derived from historically utilised medicinal herbs. The primary purpose of this research was to screen for phytochemicals and test the medicinal plants for antibacterial activity that have been historically employed. Twelve medicinal plants were chosen using the FI (frequency index) of their ethnomedicinal uses. Various established procedures were used to screen groups of phytochemical substances. Flavonoids, alkaloids, glycosides, phenols, saponins, steroids, and terpenoids were all found to be present in the plant samples, with flavonoids, alkaloids, and phenols being the most numerous. Most of the therapeutic plants studied have been shown to have secondary metabolites. The discovery provides scientific proof for the usage of these traditionally used therapeutic herbs.

1. Introduction

The emergence and spread of drug-resistant microbes has threatened the activity of available drugs and remains the major cause of treatment failure. The burden of morbidity and mortality has been inclined towards developing countries due to the increased prevalence of risk factors associated with economic transition. Antibiotics that were once thought to be miracle cures are now unable to treat resistant bacteria. Numerous multidrug-resistant microbes have been identified as virulently dangerous bacteria. Over recent years, the number of new approved antimicrobial medicines has dropped greatly, and the supply of effective antimicrobials is anticipated to run out shortly. Traditionally used medicinal plants represent the ancient and remain an indispensable source of novel and effective pharmaceutical products. The capacity to use active substances derived from plants or their synthetic equivalents in medicine has improved with the development of phytochemistry and pharmaceutical chemistry. This is due to the fact that medicinal plants have a greater variety and novelty of chemicals than any other sources.

Africa has an immensely rich biodiversity and knowledge base in the use of plants to treat various ailments, including infectious diseases. In fact, the World Health Organization (WHO) estimates that due to their easy availability, low cost, and socio-cultural background, over 80% of the population in sub-Saharan Africa relies solely on traditional medicine derived from plants for their primary health-care needs. However, these resources have hardly been investigated scientifically. In Ethiopia, some of the

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studies presented on medicinal plants were limited to an ethno medical survey, and the results were listed with incomplete descriptions.

In various regions of Ethiopia different plant species are traditionally utilized for the treatment and prevention of both human and animal illnesses. *Justicia schimperiana* (Hochst. ex Nees), *Croton macrostachyus* (Hochst. ex Delile), *Albizia gumifera* (J.F.Gmel.) *C. A. Sm, Clematis hirsuta* Guill. *and Perr, Solanum nigrum* L, *Dodonaea angustifolia* L.f., *Crinum abyssinicum* Hochst. ex A. Rich, *Dracaena steudneri* Engl., *Pycnostachys abyssinica* Fresen, *Trichilia dregaeha* Sand, are the most commonly used medicinal plants by TMPs. Therefore, it is of paramount importance to focus on antimicrobial drug discovery from medicinal plants, particularly from those which are widely used by traditional healers for the mitigation of infectious diseases.

2. Materials and Methods

2.1 Plant Material

Based on the information from the traditional healers and evidence of traditional use value frequency index, twelve medicinal plants were selected and their plant materials were collected. The selected plant species include *Andro graphis, Phyllan thus niruri, Centella asiatica, Eclipta alba, Mimosa pudica, Mucuna pruriens, Tridax procum bens, Withania somnifera*. The collected plant samples were allowed to dry at room temperature under the shade; their identification was carried out by a botanists.

2.2 Materials, Chemicals and Reagents

Materials Beakers, conical flask, measuring cylinders (different size), glass funnels, glass stirrer, cotton wool, spatula, bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holder, refrigerator, meter rule, bottles, cabinet tripod stand, wire gauze, capillary tubes, filter paper, autoclave, UV box with UV lamp, and TLC paper.

Chemicals and Reagents Analytical standards of chloroform, methanol, n-hexane ethyl acetate (Lneos Solvents Belgium), ferric chloride, HCl, Mayer-Wagner reagent (2.5 gm of iodine is dissolved in 12.5 gm of KI2 with 250 mL of distilled water), magnesium ribbon, NaOH, sulfuric acid, potassium ferricyanide (K2Fe (CN6)), dimethyl sulfoxide (DMSO) (Mettler-Toledo India Pvt. Ltd), Mueller Hinton Broth (Thermo ScientificTM), gentamycin (Bactigen FDC Limited) and clotrimazole (Glenmark Pharmaceuticals Ltd). Jimma University Laboratory of Drug Quality (JuLaDQ), organic chemistry, and microbiology labs of Jimma University provided all the chemicals and reagents.

2.3 Extraction and Fractionation

The air-dried and pulverized plant materials were extracted with chloroform/methanol 1:1 (v/v) three times for 24 hours each. The extracts were concentrated using a rotary evaporator at a temperature of 40°C to obtain crude extracts, which were subjected to phytochemical screening and antimicrobial evaluation. The crude extracts were suspended in water and further partitioned successively with n-hexane, chloroform, and methanol. Each fraction of the plant extracts was then concentrated using rotary evaporator; scanted and dried by putting it in warm mental mantle using desiccator to remove the solvent residue based on previous studies.

2.4 Phytochemical Screening

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols.

2.4.1 Test for Tannins

About 200 mg of the plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

2.4.2 Test for Alkaloids

The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

2.4.3 Test for Saponins

About 0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

2.4.4 Test for Flavonoids and Glycosides

200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 mL of distilled water and NaOH to 0.5 mL of crude extract, the formation of a yellowish color indicated the presence of glycosides.

2.4.5 Test for Steroids

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

2.4.6 Test for Terpenoids

The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

2.4.7 Test for Phenols

About 1 mL of the extract was combined with three drops of FeCl3, and 1 mL of K2Fe (CN6). The formation of greenish-blue forms confirmed the presence of phenols.

2.4.8 Thin Layer Chromatography (TLC) Test

Thin layer chromatography was performed on TLC plate (aluminum silica gel pre-coated with layer thickness of 0.2 mm) using hexane/ethyl acetate mixtures (8:2) as an eluent. Spots were applied using capillary tube 1.5 cm from the bottom marked by a line ruled using a pin. The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which was covered immediately. When the solvent reaches the top of the plate, the plate was removed, marked and dried. The number of the spots was detected under UV at 254 and 366 nm wavelengths and spraying with spotting reagent, using iodine vapour 26,27,29

3. Results

3.1 Phytochemical Screening

All selected plant extracts were presented with notable positive phytochemical results (Table 1), which were evidenced with remarkable color changes. Flavonoids, alkaloids and phenols were the most abundant classes of compounds in majorities of the screened plants. Flavonoids were exhibited highly positive with significantly visible color change in *Andro graphis, Phyllan thus niruri, Centella asiatica, Eclipta alba, Mimosa pudica, Mucu na pruriens, Tridax procum bens, Withania somnifera*. Thin layer chromatography also confirmed the presence of different phytochemical components.

Table 1 Phyto-Chemical Investigation of Selected Medicinal Plant species

Parameter	*Andro graphis*	*Phyllanthus niruri*	*Centella asiatica*	*Eclipta alba*	*Mimosa pudica*	*Mucuna pruriens*	*Tridax procum bens*	*Withania somnifera*
Alkaloids	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+
Flavonoids	+	-	+	-	+	+	+	+
Steroids	-	-	+	+	+	-	+	+
Terpenoids	-	+	+	-	+	+	-	-
Saponins	-	-	-	-	+	+	--	+
Tannin		-	-	-	-	-	-	+
Anthraquin one	-	-	+		+	+	-	-

Due to the abundance of phytochemicals found in plants, plant extracts have shown potent antimicrobial action. There has been a dearth of in-depth research on the potential of these plants as phytochemical entities and in antimicrobial treatment. Antibiotic resistance, unpleasant side effe--cts, and the exorbitant costs of synthetic drug research are turning the attention to plant-derived therapies. Potential plant species historically used to treat tropical infectious disorders, gastrointestinal infections, skin infections, and wound infections were discovered in this research. TLC data given with several spots at varying RF values revealed that most of the examined plants included multiple phytochemical classes of chemicals. These included flavonoids, alkaloids, phenols, glycosides, and steroids. The phytochemicals with the most noticeable colour changes were flavonoids, alkaloids, and phenols, which were all included in the screening process. Plants containing flavonoids, alkaloids, and phenols included *Andro graphis, Phyllan thus niruri, Centella asiatica, Eclipta alba, Mimosa pudica, Mucu na pruriens, Tridax procum bens,* and *Withania somnifera*. This result is consistent with the results of prior research.

4. Conclusion

The bulk of the chosen plant species in the present ethno medicinal survey were of the tree and herb growth habits. THs believed that these plant species were effective against a variety of illnesses, including leishmaniasis, onchocerciasis, gastrointestinal (GI), wound, and skin infections. Phenols and alkaloids were the most common groups of phytochemicals that caused colour shifts and TLC spots. The flavonoids in Andro graphis, Tridax procum bens, and Withania somnifera were prominently shown with a noticeable apparent colour shift. *Phyllan thus niruri, Centella asiatica, Eclipta alba, Mimosa pudica,* and *Mucu na pruriens*, all contain alkaloids, however they are not the most common kind of component in these plants. *Andrographis, Tridax procum bens, and Withania somnifera* also included the phytochemical flavionods. The results show promise for the future of antibacterial medication development and provide credence to the traditional knowledge that relies on these plants. Further pharmacological researches are necessary to be undertaken employing various microbial strains for effective plant species. Plant species that show substantial activity should be subjected to toxicological testing, in vivo bioactivity research, and molecular characterisation.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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