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Review Article

Review On Phytochemical And Pharmacological Investigation of Syzygium Guineense Extract

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ABSTRACT

The leaves of Syzygium guineense have been founded useful for the control and cure of malaria, and performed antiplasmodial activity in vitro. Investigation of this plant was carried out to analyse antioxidant and antimicrobial properties of characterized essential oil of the leaf of Syzgium guineense (S. guineense). Essential oil obtained by hydrodistillation was identifyed by gas chromatography coupled with mass spectrometry (GC-MS). The antioxidant potential was discovered by measuring the inhibition of 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The essential oil were tested against five bacteriafor gives their inhibitory effects : "Mycobacterium bovis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus" and one fungus: Candida albicans using broth microdilution method. The GC-MS analysis revealed the presence of 46 components accounting for 92.63% of the essential oil constituents. Sesquiterpenoids (73.15%) and monoterpenoids (14.17%) were the main classes of the essential oil. Aromadendrene (6.98%), germacrene B (5.52%) and β-selinene (3.94%) were the predominant sesquiterpene hydrocarbons. The oxygenated sesquiterpenes were α-cadinol (6.68%), τ-cadinol (6.64%) and caryophyllene oxide (5.44%). Butylated hydroxytoluene (BHT) exhibited higher antioxidant activity compare to the essential oil. The essential oil revealed strong antimicrobial activities against the tested microorganism with MIC ranging between 25-100µg/mL. Results showed that the leaf essential oil of S. guineense had high amount of sesquiterpenoids (73.15%) with strong antioxidant and antimicrobial activities.

INTRODUCTION

Some species of syzygium had been reported that the antimicrobial activity of the essential oil.4-6,26

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Triterpene constituents from the leaves of S. guineense showed the most significant antibacterial activities against Escherichia coli, Bacillus subtilis and Shigelli sonnei27. The growth of Staphylococcus epidermidis, Staphylococcus aureus and Shigella dysentariae, a Yersinia enterocolitica, Salmonella sp., Serratia marcescens, Pseudomunas aeruginosa and E. coli28 was inhibited by the aqueous extracts of the leaf and the stem barks of S. guineense Syzygium aromaticum essential oil and its main component eugenol showed inhibitory activity against Candida, Aspergillus and dermatophyte species. They caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component29 .The essential oil from S. cumini leaf was reported to comparatively inhibit the growth of those bacterial strains [1-3]. Syzygium guineense showed presence of secondary metabolites such as flavonoids, tannins, saponin, alkaloids and cardiac glycosides by the phytochemical screening

Taxonomy[9]

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Myrtales
Family	Myrtaceae
Genus	syzygium Species

test of leaf extracts of S. guineense. These metabolites are detained to be subjected for traditional medicinal uses of the plant or its potential as source of bioactive compounds to be used in drug discovery . For instance, methanolic extract of leaves of Syzygium guineense on mice test showed its potential for treatment of snake bites and as antidiabetic agent whereas its ethanolic extract showed anti-inflammatory, analgesic activities and antibacterial activities[4] .

Fig 2- Syzygium guineens fleaves

Plant Source

Syzygium guineense (Myrtaceae) is an odorous species native to the wooded savannahs and tropical forests of Africa. This short-trunked tree grows widely in northern Benin. Its wild, oval fruits are edible. It is included among the African plant species that are active against malaria. The bark of S. guineense is used in traditional medicine to treat gasto-intestinal upsets and diarrhoea[5-11].

METHODS AND MATERIALS

2.1 Collection and identification of the plant

The roots of Syzygium guineense were collected in November, 2016 from Shonie town, Hadya Zone, South Nation Nationalities Peoples' Region (SNNPR), Ethiopia. The area is located about 340 km south of Addis Ababa, and 125 km West of Hawassa University, Ethiopia. The plant species was identified and authenticated by botanist Reta Regassa, Department of Plant Science, Hawassa Teachers' Training College, Ethiopia. Fresh green leaves of Syzygium guineense were collected in August 2017 from Suleja, Niger State, Nigeria. The plant was identified and authenticated by a taxonomist at the Herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, where a voucher specimen with number NIPRD/H/6644 was deposited

2.2 Extraction procedure Fresh matured leaves of S. guineense

(Wild) D.C. were collected, washed, air dried under shade and grinded using mortar and pestle to coarse powder. A total of 400 g powder was extracted by maceration for 72 h, filtered with wattman filter paper (150 mm size), and the residue extracted further two times by maceration with the same duration (72 h each). Finally, the combined filtrate was kept in an oven (40°C) for concentration. Further drying process was carried out in a desiccator for removal of water. Finally, the dried extract was maintained at −4 °C till the beginning of the actual experiment.

2.3 Phytochemical screening tests

Phytochemical screening tests were carried out on the crude extracts of n-hexane, dichloromethane/methanol (1:1) and methanol using standard procedures reported in literature to detect the presence of secondary metabolites namely ~ 3106 ~ Journal of Pharmacognosy and Phytochemistry steroids, terpenoids, saponins, flavonoids, tannins alkaloids, phenols and glycosides.[12,13-15]

2.3.1 Test for steroids:

Two milliliter of acetic anhydride was added to 2 ml of extract that was dissolved in methanol and then 2 ml of H2SO4 was added into the test tube containing the mixture Appearance of a blue-green ring indicates the presence of steroids.[13]

2.3.2 Test for terpenoids:

Five millilitre of extract that was dissolved in methanol was mixed with 2 ml of chloroform. Then 3 ml of concentrated H2SO4 was carefully added into the test tube containing the mixture in order to get a layer (Salkowski test) Appearance of a reddish-brown coloration indicated the presence terpenoids.[13]

2.3.3 Test for saponins:

0.2 g of crude extract was dissolved in 5 ml of water in test tube and shaken vigorously for 15-minute Formation of 1 cm layer of foam indicates the presence of saponins.[14]

2.3.4 Test for flavonoids:

3 ml of extract dissolved by methanol was treated with 3 drops of sodium hydroxide solution (Alkaline Reagent Test). Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids [15]

2.3.5 Test for tannins:

0.2 g of crude extract was boiled in 20 ml of water in a test tube. The solution was filtered and 4 drops of 0.1% ferric chloride (FeCl3) was added into the test tube. Appearance of a brownish green or blue-black coloration confirmed presence of tannins.[12]

2.3.6 Test for alkaloids:

ne ml of 1% HCl was added into a test tube containing 3 ml of the extract. The mixture was heated for 20 min, cooled and filtered. To 1 ml of the filtrate, 0.5 ml Dragendorff's reagent was added . A red precipitate that confirmed the

presence of alkaloids was observed at the end of the test.[15]

2.3.8 Test for glycosides:

Two milliliter of chloroform was added in 2 ml of extract dissolved by methanol and then 2 ml H2SO4 was added carefully and shaken gently. A color change from orange to reddish brown at interface was observe [14]

Table no 1 Detail of Phytoconstituents screening of Syzygium guineense plant Leaves Extract

Sr. No	Phytoconstituents	Test/Reagent	Result
1	Alkoloids	Wagners	+
2	Tannins	KOH	+
3	Flavonoids	Shinoda Test	-
4	Terpenoids	Salkowski Test	-
5	Anthraquinone glycoside	Borntragers Test	+
6	Cardiac glycosides	Keller-kiliani Test	+
7	Saponins	Frothing Test	+

3. Pharmacological properties

Triterpenes, including 6-hydroxyasiatic acid, oleanolic acid and ursolic acid, account for the antibacterial activity of leaves of some Syzygium species[16,17] . Flavonoids and tannins isolated from S. guineense reported with analgesic and anti-inflammatory activities.[18] Flavonoids are also known for their antiallergic, antimicrobial and anticancer properties[19] . Study also indicates that the bark extracts of S. guineense possess antioxidant properties and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants[20] . These findings suggest that antioxidant properties of S. guineense extracts could be attributed to phenolic compounds revealed by phytochemical studies[24] . Triterpenes isolated and characterized from the plant are biologically active on bacteria, showed activity against strains of Salmonella E., Shigella D., Shigella F., E. coli and Enterobacter[25] . Leaves of S. guineense produce antibacterial activity against some organisms.[26]

3.1 Antimicrobial effect

The inhibitory activities of the leaf essential oil of S.guineense against bacteria and fungi were evaluated using broth micro-dilution method. The S. guineense leaf essential oil consisted of monoterpenes, sesquiterpenes, non-terpenes, diterpenes and triterpenes. Sesquiterpenoids (73.15%) consisting of oxygenated sesquiterpenes (40.12%) and sesquiterpene hydrocarbons (33.03%) were the predominant classes among the identified components. Other classes of the essential oil constituents were monoterpenoid (14.17%), oxygenated monoterpenes (8.11%) and monoterpene hydrocarbons (6.06%). Among the essential oils obtained from Syzygium species, S. samarangense resembled the present report based on the pattern of oxygenated sesquiterpenes (40.2%), sequiterpene hydrocarbons (27.2%), oxygenated monoterpenes (11.1%), monoterpene hydrocarbons (6.6%) and oxygenated diterpenes (0.7%)38 . The monoterpenes found in S. guineense essential oil included acyclic monoterpenes (citral), monocyclic monoterpenes (D- limonene) and bicyclic monoterpenes (α-pinene, β-pinene, myrtenal and 1,8-cineole). The sequiterpenes being the most abundant constituent comprised of acyclic sesquiterpenes (Neryl- (S)-2-methyl butanoate, fernesyl acetone and fernesyl acetate); monocyclic sesquiterpenes (germacrene B and isoshybunone), bicyclic sesquiterpenes (α-cadinol, τcadinol, δ-cadinol, caryophyllene oxide, β-selinene and 6- isopropenyl-4,8a-dimethyl- 1,2,3,5,6,7,8,8aoctahydronaphthalene-2,3-diol) and tricyclic sesquiterpenes (aromadendrene, (-)- globulol, alloaromadendrene, (-)-β-bourbonene and α-cedrene). Acyclic diterpene alcohol (phytol) and acyclic triterpene (squalene) were among the terpene constituents present in the essential oil.

3.2 Method of Antibacterial Evaluation

All the antibacterial tests were conducted at Oromia Public Health Research, Capacity Building & Quality Assurance Laboratory,

Adama, Ethiopia. The in-vitro antibacterial activity of SyG-Ag NPs was evaluated using Agar disc-diffusion method against selected one Gram positive bacterial strain (Staphylococcus aureus) and three Gram negative pathogenic bacterial strains (Escherichia coli, Pseudomonas aeruginosa and Enterobacter aerogenes). Prior to antibacterial activity test, the bacterial strains were cultured in nutrient broth for 24 hrs to obtain logarithmic growth phase of the test bacteria. A standardized inoculum of the bacteria is swabbed onto the surface of Mueller- Hinton Agar (MHA) plate. The actively growing bacterial cultures of 1.3×108 CFU/mL concentration were inoculated/spread onto the25 and 50 µg/µl) of the synthesized nanoparticles were added to the respectively labeled wells. The antibiotic discs of 6 mm diameter were applied to agar surface using forceps with gentle pressure and then impregnated with the dissolved extract. Chloramphenicol disc was used as a positive control while DMSO was taken as negative control. The plates were incubated at 35 ±2°C in an ambient air incubator for 18-24 hrs. The antibacterial activity was evaluated in terms of zone of inhibition, measured to the nearest millimeters (mm) using a ruler and recorded. MHA plate (turbidity was adjusted with TSB to match 0.5 McFarland standard). The nanoparticles extract was prepared with four different concentrations in Dimethyl Sulfoxide. Four concentrations 6.25, 12.5

3.3 Antioxidant activity

The antioxidant activity of S. guineense was tested using DPPH free radical scavenging method by comparing with the efficacy of an established antioxidant agent BHT. DPPH antioxidant assay involves the abstraction of hydrogen by the free radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) from the antioxidant molecule, leading to decolorization of the DPPH. The degree of decolorization of DPPH is a measure of the free radical scavenging capacity of the sample under study. In this study, BHT exhibited higher antioxidant activity compared to the essential oil. However, the scavenging potential of S. guineense oil was concentration dependent. The essential oil can play a vital role in alleviating oxidative stress conditions in different diseases such as liver, renal, neurodegenerative and cardiovascular diseases, cancer, diabetes as well as ageing processes37 .

CONCLUSION

In this study, phytochemical screening tests were carried out on n-hexane dichloromethane: methanol (1:1) and methanol extracts of roots of Syzygium guineense. The results revealed the presence of steriods, terpenoids, saponins, flavonoids, tannins, alkaloids, phenol and glycosides in the dichloromethane: methanol (1:1) and methanol extracts whereas only terpenoids and steroids were detected in the nhexane extract. The presence of these metabolites could be responsible for medicinal use of the Syzygium guineense The GC-MS analysis of S. guineense leaf essential oil revealed the presence of 46 components accounting for 92.63% of the essential oil constituents many of which are biologically active. Some of the compounds have antioxidant, anti-inflammatory, antifungal and antimicrobial properties. S. guineense essential oil exhibited strong antimicrobial activities against the tested microorganisms with MIC range of 25- 100µg/mL.

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