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PRELIMINARY PHYTOCHEMICAL DETECTION, QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS, TOTAL PHENOLS AND ANTIOXIDANT ACTIVITY OF LEAVES OF SYZYGIUMMALACCENSE(MYRTACEAE)

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***ABSTRACT:** Preliminary phytochemical screening is the key step in finding the chemicals that lead to the isolation of lead compounds of medicinal importance. Syzygium malaccense is a large woody climbing shrub that belongs to the Myrtaceae family. Its fruits and leaves are very active from a medical point of view and are used in many traditional medicinal systems. The main objective of this study is to identify and understand the bioactive chemical components of leaf extracts by subjecting the leaf powder to soxhlet extraction in different solvent systems. The phytochemical screening showed the presence of proteins, coumarins, saponins, flavonoids, tannins, and phenolic compounds etc. Total phenolic contents in the methanolic and Aqueous extract of S.malaccenseleaves were found to be 106.21 ± 10.85mg/g and 84.09 ± 6.32 mg/g equivalent to gallic acid respectively and flavonoid contents in the methanolic and Aqueous extract of S.malaccenseleaves were found to be 80.65 ± 7.46 mg/g and 58.54 ± 9.21 mg/g equivalent to rutin respectively.Syzygium malaccense leaves methanolic extract has been shown to have high antioxidant activity and powerful ability to scavenge free oxygen radicals, plus the plant's IC_{50} was nearly equivalent to the standard antioxidant that justified its use in medications. traditional and could represent a good candidate for additional biological and chemical analyzes, and can also be subjected to the isolation of therapeutically*

active compounds with antioxidant activity and also for additional pharmacological evaluations.

KEYWORDS:Syzygium malaccense, Preliminary phytochemical screening, Total flavonoids, Total phenols, Antioxidant activity.

INTRODUCTION:

Medicines of plant origin have been used throughout the world in traditional medicine for the treatment of various diseases. According to a survey by the NCI, USA, 61% of the 877 new small molecule chemical entities were introduced as drugs[1]. Plant species remain a rich source of many new biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties[2]. Therefore, there has been a renewed interest in phytomedicine in the last decade and many species of medicinal plants are currently being tested for pharmacological activities. Herbal preparations are most often used to prevent and treat various diseases around the world. In developing countries, the World Health Organization (WHO) estimates that around 80% of the population depends on herbal preparations used in their traditional medicine system and as basic necessities for primary human health care. Detailed research and documentation of plants used in local health traditions and ethnopharmacological evaluation to verify their efficacy and safety can lead to the development of invaluable herbal medicines or the isolation of compounds of therapeutic value [3,4].

The plant synthesizes a wide variety of chemical compounds, which can be classified by chemical class, biosynthetic origin, and functional groups in primary and secondary metabolites. Primary metabolites constitute the physical integrity of the plant cell and participate in the primary metabolic process of construction and maintenance of living cells. Secondary metabolites are not considered vital for the immediate survival of the organism that produces them and they are not an essential part of the process of building and maintaining living cells. With the development of natural product chemistry, the potential for chemotaxonomy is becoming increasingly apparent. The application of chemical data to systematics has received a great deal of attention from a great number of biochemists and botanists during the last three decades.

The natural constituents of plant origin can be derived from any part of the plant, such as bark, leaves, flowers, roots, fruits, seeds, etc.[5] In other words, any part of the plant can contain active

components. The beneficial medicinal effects of plant materials are typically derived from combinations of by-products present in the plant such as alkaloids, glycosides, resins, volatile oils, gums, tannins, etc.[6,7]

Figure 1: Syzygium malaccense plant

Syzygium malaccenseis an evergreen tree with an expanded but cone-shaped crown; usually grows from 5 to 20 meters tall. The evergreen leaves are opposite, soft leathery and dark green - the flowers are purplish-red in color and form a carpet after falling under the tree. The fruit is oblong in the shape of a pear with dark red skin and white pulp; sometimes it has no seeds. The fruit is oblong in shape and dark red, although some varieties have white or pink skin. The pulp is white and surrounds a large seed[8, 9].

MATERIALS AND METHODS:

Plant Material

Fresh leaves of S. malaccense were obtained from the Nashik, Maharashtra and authenticated at the Botanical survey of India, Pune, where a specimen has been deposited.(Ref no. BSI/WRC/100-1/Iden.cert./2018/92)

Preparation of the Plant Extracts

100g of dried leaves powder was extracted with methanol and water using soxhlet apparatus. Both extracts were concentrated to a small volume by rotary evaporator. The solvent fraction containing the bioactive compounds was used for further studies[10].

Phytochemical Screening of extracts

Aqueous and methanolic extracts were subjected to preliminary phytochemical screening using standard methods for detection of the various constituents.

Determination of total phenol content

The total phenolic content in the methanolic and ethanolic plant extracts was determined using the spectrophotometric method[11] with some modifications. In the analysis, 1 mg / ml aqueous solutions were prepared for methanolic and ethanolic extracts. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% FolinCiocalteu reagent dissolved in water and 2.5 ml of 7.5% aqueous NaHCO3 solution. The samples were then incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using a spectrophotometer at wavelength = 765 nm. Samples were prepared in triplicate for each run and the mean absorbance value was obtained. The same procedure was repeated for the gallic acid standard solution and the calibration line was constructed. Based on the measured absorbance, the equivalent gallic acid concentration expressed in terms of (mg GA / g extract).

Determination of flavonoid content

The total flavonoid content was determined from the Rutin calibration curve and was expressed as milligrams of Routine equivalent per gram of extract (mg RU / g extract). The total flavonoid content was determined according to the procedure of Chang et al.[12], validated by Nugroho[13] with some modifications using the routine as a reference standard. The rutin (100 mg) was dissolved in 10 ml of distilled water and diluted to 100 ml. Subsequently, the stock solution was diluted to give a range of concentrations (5, 10, 20, 40, 100 µg / ml). of each solution (0.5 ml) was mixed with 3 ml of methanol, 0.2 ml of 10% AlCl3, 0.2 ml of 1M potassium acetate and 5 ml of distilled water, and then incubated at temperature environment for 30 minutes.

Antioxidant activity

The free radical scavenging activity of the aqueous and methanolic extract of Syzygium malaccense was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH)[14]. An aliquot of (0.05, 0.1, 0.5, 1.0, 1.25 and 1.50 mg / ml) of aqueous and methanolic extract of Syzygium malaccense was mixed in tubes containing 3 ml of methanol and 0.5 ml DPPH 1mM separately. The mixtures were left in the dark for 30 min at room temperature to check the reaction of the extract on the DPPH radical. The absorbance of the mixture was read spectrophotometrically at 517 nm, using ascorbic acid as a reference. The ability of the extract to remove the DPPH radical was determined by the given equation: Percentage of inhibition = A0-A1 / A0 × 100

where A0 is the absorbance of the control and A1 is the absorbance of the extracts / standards. Then% inhibition was plotted versus concentration and IC_{50} was calculated from the graph. The experiment was repeated three times at each concentration[15].

RESULT AND DISCUSSION:

Preliminary phytochemical screening

The phytochemical analysis conducted on leaves extracts of Syzygium malaccense revealed the presence of proteins, coumarins, saponins, flavonoids, tannins, and phenolic compounds (Table 1). These phytochemical compounds are known to support bioactive activities in Syzygium malaccense leaves and thus responsible for the antioxidant activities of this plant extract used in this study.

Constituents	Methanolic extract	Aqueous extract
Alkaloids	-	-
Proteins	+	+
Coumarins	+	+
Glycosides	-	-
Phenolic compounds	+++	+
Tannins	++	++
Flavonoids	++	+
Terpenoids	+	-
Saponins	+	+
Sterols	+	-

'+++' High concentration, '++ 'intermediate concentration, '+ 'low concentration, '-' absent

Table 1: Phytochemical screening of methanolic and aqueous extract of Syzygium malaccense leaves.

Estimation of total phenolic and flavonoid contents

Quantitative estimation of total phenolic and flavonoid contents for leaves extracts of Syzygium malaccensestudied is presented in Table 2.

Estimation	Methanolic extract	Aqueous extract
Total phenolic	106.21 $\pm$ 10.85 mg/g	84.09 $\pm$ 6.32 mg/g
Flavonoids	80.65 $\pm$ 7.46 mg/g	58.54 $\pm$ 9.21 mg/g

Table 2:Estimation of total phenolic and flavonoid contents

Determination of antioxidant activity

The scavenging effects of leaves extracts of Syzygium malaccense were found to be concentration dependent. Both extracts reacted with the stable free radical DPPH and discolored its solution. The percentage inhibition by methanolic extract at different concentrations of 0.05, 0.1, 0.5, 1.0, 1.25 and 1.50 mg/ml were found to be 34.12, 38.99, 51.36, 74.10, 86.26 and 93.98% respectively whereas, in comparably doses, that of the standard ascorbic acid were found to be 37.91, 45.24, 52.98, 78.51, 88.12 and 97.12% respectively. The standard ascorbic acid presented a high scavenging effect of 97.12% at the concentration of 1.50 mg/ml. The IC_{50} value of methanolic extract of s. malaccense was found to be 250 μg/ml, which is comparable to the IC_{50} of standard ascorbic acid 220 μg/ml (Figure 2).

Concentrations (mg/ml)	Percentage inhibition (%)		
	Methanolic extract	Aqueous extract	Ascorbic acid
0.05	34.12	28.23	37.91
0.1	38.99	34.75	45.24
0.5	51.36	48.39	52.98
1.0	74.10	69.96	78.51
1.25	86.26	79.38	88.12
1.50	93.98	88.67	97.12

Table 3:% Inhibition by extract at different concentration

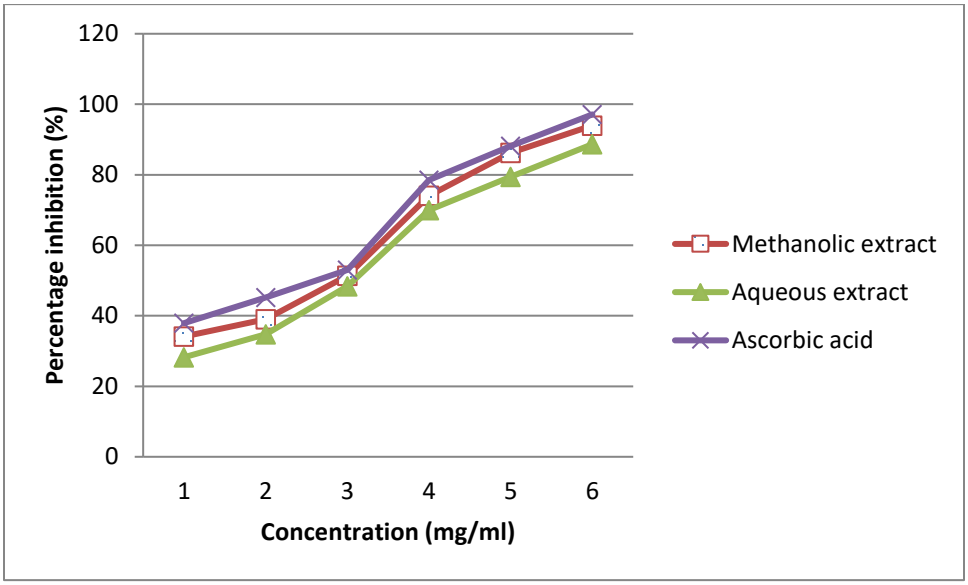

Figure 2: Graph showing DPPH free radical scavenging activity of extracts of S. malaccense

CONCLUSION:

Medicinal plants have the highest therapeutic efficacy in the pharmaceutical field[16]. Medicinal plants have been used to treat so many diseases and their medicinal role for these plants has a side product and they have identified bioactive compounds. The medicinal value of these plants lies in certain chemicals that produce a defined physiological action on the human body[17]. Phytochemical examination showed that Syzygium malaccense leaf extract contains a mixture of phytochemicals such as proteins, coumarins, saponins, flavonoids, tannins, and phenolics. The quantitative screening of total flavonoids and total phenol indicated that the methanolic extract of the leaves has the highest content of flavonoids and phenols.The results of the antioxidant study indicated that flavonoids and phenolic compounds are responsible for the washing and antioxidant activities of the aqueous extract and methanolic from S. malaccense leaves. The methanolic extract showed high IC_{50} values that are comparable to standard ascorbic acid.

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