



RESEARCH ARTICLE

Phytochemical and Antioxidant Screening of leaf extract of *Syzygium cumini***Ashwani Kumar, Manoj Kalakoti**Institute Of Biotechnology, G.B.Pant University of Agriculture & Technology, Patwadangar, Nainital,
Uttarakhand**Manuscript Info****Manuscript History:**Received: 29 November 2014
Final Accepted: 10 December 2014
Published Online: January 2015**Key words:**

Phytochemicals , Antioxidants

Corresponding Author*Manoj Kalakoti****Abstract**

Syzygium cumini is commonly known as *Jamun*. The investigation of this plant aims to assess the phytochemical and antioxidant content of the methanolic leaf extract of locally available *Syzygium cumini*. Different tests are performed to find the phytochemicals and antioxidant that are present so as to subject the plant for further medicinal uses. These chemical constituents are mainly responsible for various biological activities. From the phytochemical and antioxidant studies conducted it was found that there is the presence of alkaloids, steroids, saponins, cardiac glycosides, carbohydrate, protein, tannin and phenol in the plant sample taken from the forest of Patwadangar region during the month of July. Firstly the collection of leaves, they were dried and crushed into a powdered form. Finally an extract was prepared using methanolic solvent. After this the phytochemical and antioxidants analysis was performed to find out the above mentioned chemical constituents in *Syzygium cumini* from Patwadangar.

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INTRODUCTION

The genus of *Syzygium cumini* (L.) commonly known as "jamun" is widely used in Ayurveda and other Indian folk medicines for the treatment of diabetes mellitus (high blood sugar levels). A fairly fast growing species, it can reach heights of up to 30 m and can live more than 100 years. Its dense foliage provides shade and is grown just for its ornamental value. At the base of the tree, the bark is rough and dark grey, becoming lighter grey and smoother higher up. The wood is strong and is water resistant. Because of this it is used in railway sleepers and to install motors in wells. It is sometimes used to make cheap furniture and village dwellings though it is relatively hard to work on. The leaves which are an aroma similar to turpentine, are pinkish when young, changing to a leathery, glossy dark green with a yellow midrib as they mature. The leaves are used as food for livestock, as they have good nutritional value.

classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Myrtales
Family:	Myrtaceae
Genus:	<i>Syzygium</i>

PHYTOCHEMICAL ANALYSIS:

“Phytochemistry is study of phytochemical found in plants describing the extraction, isolation, purification, identification and structural elucidation of various plant secondary metabolites”

1. Extraction of phytochemicals.

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. This product contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed.

Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation.

Extract preparation:

First step is the collection of leaves after which the leaves are first washed in running water for 15 minutes. After washing let the leaves dry on the paper sheets(3-4 days). After the leaves are dried, grind the leaves in grinder and filter the leaf extract by cloth and store the filtered part of the leaf sample.

Methods of extraction:

- Solvent extraction by soxhlet apparatus:
 - Alcoholic extraction (by methanol)

methanol Solvent extraction by soxhlet method:

1. Weight the leaves (5g).
2. Put the content in thumble and set up the soxhlet apparatus.
3. Add 150 ml of the solvent.
4. After the completion of 10-12 cycles take the solvent.
5. Evaporate in oven so as to get dry extract.

**The basic parameters influencing the quality of an extract are:**

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon:

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants.

The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted.

Methanolic Solvent**Solvents used for active component extraction:****Methanol**

- Anthocyanins
- Terpenoids
- Saponins
- Tannins
- Xanthoxylines
- Totarol
- Quassinoids

Phytochemical Screening**Qualitative determination:**

Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug as per the standard methods.

Materials, Instruments and Chemicals:

Different extracts of all samples, different chemical reagents, test tubes (Borosil), test tube stand. Spatula etc.

Procedure: Few mg of plant extract was taken in different-different test tube and required amount of solvent added in each test tube. Then it was subjected to different-different chemical reagents and tests and observation was noted.

1. Test for Carbohydrate:

- a. **Molisch's test:** Treated the extract with few drop of alcoholic α -naphthol, added 0.2 ml of concentrated sulfuric acid slowly through side surface of test tube, purple to violet color ring appears at the junction. (α -naphthol : 10g of α -naphthol in 100ml of 95% alcohol).

- a) **Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

9. Detection of phytosterols

a. **Salkowski's Test Extracts** : were treated with chloroform and filtered. The filtrates were treated with few drops of Conc.Sulphuric acid, shaken and allowed to stand Appearance of golden yellow colour indicates the presence of triterpenes.

RESULT:

Qualitative Phytochemicals Screening

Different qualitative chemical tests were performed for establishing the profiles of the extracts for their nature of chemical composition and for identification of various phytoconstituents.

The result of the phytochemical analysis of the plant *Syzygium cumini* is as follows:

Table no 1 :

TEST	METHANO L EXTRACT
Molish's test	+
Benedict test	+
Fehling test	+
Legal test	-
Ferric chloride test	-
Lead acetate test	+
Wagner	+
Hager	+
Mayer	+
Biuret test	-
Xanthoprotein test	+
Shinoda test	-
Alkaline reagent test	-
Salkowaski test	-

Antioxidant Activity:

DPPH Radical Scavenging Activity

The free radical scavenging activities of the different crude extracts of leaves of the plant on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand Williams. Inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [1 - (\text{ABS}_{\text{sample}} / \text{ABS}_{\text{control}})] \times 100$$

Where $\text{ABS}_{\text{control}}$ is the absorbance of the control reaction (containing all reagents except the test material) and $\text{ABS}_{\text{sample}}$ is the absorbance of the sample material. Then percent inhibitions were plotted against respective concentrations. IC_{50} values were calculated as the concentration of

each sample required to give 50% DPPH radical scavenging activity from the graph. Tert-butyl-1- hydroxytoluene (BHT) and Ascorbic acid were used as positive control. The experiment was performed thrice and the result was expressed as Mean±Standard Error of Mean (SEM) in every case.

Statistical Analysis

The data represents mean ± SEM results were analysed statistically by ANOVA followed by Dunnett's 't' test using computer fitted Graph pad prism software student's version 5.0. The difference was considered significant when $P < 0.05$.

QT _j	TT _j	Conc _j	OD _j	IT _j
40ul	2:41	360	00.290	2:11
80 ul	2:43	335	0.254	2:12
120 ul	2:45	362	0.281	2:13
160 ul	2:46	507	0.393	2:14
200 ul	2:48	630	0.490	2:15

In vitro Antioxidant Activity:

In vitro antioxidant studies revealed that Methanolic extract of *Syzygium cumini* showed the potent scavenging activity by DPPH method with the IC₅₀ value of 0.584 ± 4.0 µg/ml. The concentration of the plant extract needed for 50% scavenging (IC₅₀) of DPPH was found to be 106.34µg/ml. Two positive controls were used- Butyl hydroxyl toluene (BHT) and Ascorbic acid (AS) for which the IC₅₀ values were found to be 1.126 µg/ml for BHT and 1.150 µg/ml for Ascorbic acid (AS) respectively. The results for the in vitro antioxidant activity were tabulated in the table no 2

Table no 2: In vitro antioxidant activity of Methanolic Extract of Leaves of *Syzygium cumini*

Extract/ Compound	Antioxidant activity IC ₅₀ ug/ml of DPPH Assay
MEO	0.585 ± 4.1
BHT	1.125 ± 0.40
AS	1.140 ± 0.003

Table no 2: Values are expressed as mean ± SEM in three replicates. Positive control used BHT= Butyl hydroxyl toluene; AS= Ascorbic acid. Sample as Methanolic extract of *Syzygium cumini* and negative control used as DPPH= 1,1-diphenyl-2-picrylhydrazyl

CONCLUSIONS

There are many herbal plants in the world but the *Syzygium cumini* (Jamun) is considered to be the queen of herbs due to its greater medicinal values. It is well documented in the Hindu mythology about the Tulsi. Considering the health beneficial effects of Tulsi our ancestors in India insisted to plant a Tulsi sapling in everyone's house. Keeping the various medical benefits in view, investigations are called for to be attempted towards purifications of Tulsi components and their characterization in terms of chemical natures and bio-pharmacological activities. Probably, such natural components might prove to be potentially beneficial but comparatively less toxic. Eventually, plants belonging to *Syzygium* genus could contribute a lot towards economy and healthy problem.

Acknowledgements

The authors are greatly thankful to the Director of IBT Patwadangar, Nanital and IBT scientific staff for their encouragement and support during the work.

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