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Short Communication

The Effect of Different Extraction Methods on Antioxidant Capacity and Phytochemical Screening of *Syzygium cumini* Seeds

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ABSTRACT

Objective: *Syzygium cumini* has been used for centuries in traditional medicine for various athological conditions. In the present study, extract yield, phytochemical constituents and *in-vitro* antioxidant assay ethanolic extracts of *Syzygium cumini* seeds was investigated. **Methods:** Extraction techniques like ultrasound microwave assisted solvent, maceration and soxhlet were used for extraction. The extracts were evaluated for potential antioxidant activities by using DPPH and ABTS assay. **Results:** Our results revealed that extract yield, chemical composition of the extracts and antioxidant activity of the *Syzygium cumini* extract varied with the extraction process. The results exhibited highest extraction yield and flavonoids, alkaloids and glycoside in ultrasound microwave assisted solvent extraction followed by soxhlet extraction and least in maceration extraction method respectively. All extraction methods showed free radical scavenging potential, ultrasound microwave assisted solvent extraction exhibited significant scavenging potential having an IC_{50} value of 69 ± 8.11 µg/mL for DPPH and 87 ± 5.86 µg/mL for ABTS. Naringin is used as a reference standard antioxidant agent. **Conclusion:** These results revealed that the ultrasound microwave assisted solvent extraction of *Syzygium cumini* seeds can be a rich source of antioxidants containing flavonoids, alkaloids and glycoside. The antioxidants chemical compounds present in *Syzygium cumini* seeds have various beneficial effects in the phytopharmaceuticals industry.

Key words: *Syzygium cumini,* Extraction, Phytochemical Screening, Antioxidant, DPPH, ABTS.

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INTRODUCTION

For centuries, natural products from flora and fauna have been used by the mankind as a foremost source of remedy against various disease and disorders. Natural products played a significant part in complementary and traditional medicine systems, like Ayurveda, Unani and Chinese which are still part of our health care system. As per the statistical date published by the World Health Organization (WHO), still 75% of the global population directly or in directly relies on natural products based medicine for primary health care. Natural products have the proficiency to manufacture various secondary metabolites, which act as a defensive shield against free radicals.[1] Literature survey revealed that high concentrations of these secondary metabolites especially polyphenolic compounds are possessing high antioxidant activity compared to the other secondary metabolites.[2] Due to this property natural products can be good candidates for the development of novel functional foods with promising effects on human health.[3]

Therefore, it's clear that natural products keep on representing rich mostly untapped resources for the discovery of the drugs with potential claim for the management of contemporary diseases of humans.[4]

Syzygium cumini (L.) also known as jambolan is an evergreen tree distributed throughout the Asia and Africa countries, belonging to the family Myrtaceae. All parts of the *Syzygium cumini* are used in complementary and traditional system of medicine in India.[5,6] It has been reported that in some parts of Asia, the tree is mostly planted in the Hindu temples because they consider sacred to Lord Krishna and is also respected by the people of the Buddhist faith.[7] Around the globe several herbal formulations were also prepared in as individual or in combination with this natural products which exhibits significant antidiabetic activity. Literature survey revealed that different parts of the *Syzygium cumini* were used as an antioxidant, anti-inflammatory, neuroprotective, anti-bacterial, antifungal anti-HIV and radio protective activities.[8] Phytochemical chemical constituents present in *Syzygium cumini* such as Jambolan are known to have compounds containing rich source of glucoside, flavonoids, terpenoids, sugars and polyphenols.[7] It has been reported that the quantity of phenolic compounds present in plant extracts were mostly depending on the polarity of solvents used in the extraction process.[9]

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Reactive oxygen species play an important role in the pathogenesis of various diseases and disorders. Literature survey revealed that intake of natural products is interrelated with a reduced risk of diseases/disorders and these encouraging properties of the natural products have been partly attributed to the components that possess antioxidant capacities. Antioxidants isolated from the various natural products resources have turned out attention in the researcher as an alternative to synthetic antioxidants due to safety concerns and limitation of usage.[10]

In this study, the extraction of *Syzygium cumini* is done by using various extractions techniques which are soxhlet, maceration and ultrasound microwave assisted solvent extraction. However, this folklore extraction techniques used to achieve these nature of products have several limitation, they are time consuming, laborious and have low selectivity. To overcome these circumstances, a new extraction method which is ultrasound microwave assisted solvent extraction is being studied.

MATERIAL AND METHODS

Reagents and standards

2,2-diphenyl-1-picryl-hydrazyl-hydrate(DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium persulfate were obtained from sigma-aldrich (United states of America). Naringenin, Hydrochloric acid, Sulphuric acid and Nitric acid were purchased from Sisco Research Laboratories Pvt. Ltd. (India). Rest all reagents and chemicals used in the present experiment were of analytical grade.

Extraction techniques

Soxhlet extraction (SxE)

Syzygium cumini seeds were coarsely powdered, to provide a greater surface area. Then the material was filled in the cellulose thimble. Methanol was used as a solvent for extraction. The process was run for a total of 8 hours. Afterwards, the mixture was filtered and the methanol was recovered under reduced pressure using Buchi Rotavapor® R-210. Then extract was kept in desiccator for further use.

Maceration extraction (ME)

Coarsely powdered seeds of *Syzygium cumini* were kept in contact with methanol in a stoppered container for 24 hrs period with frequent agitation. This cycle was repeated several time in order to exhaust the plant material. Then the extractive solutions were pooled, filtered and concentrated to dryness under reduced pressure using Buchi Rotavapor® R-210. Then extract was kept in desiccator for further use.

Ultrasound microwave assisted solvent extraction (SE)

Coarsely powdered seeds were kept in methanol and exposed to ultrasound frequencies gradually ranging from 20 kHz to 50 kHz for one hour. After the sonication, the mixture is filtered through a Whatman no. 1 filter paper, the filtrate was recovered under reduced pressure using Buchi Rotavapor® R-210. Then extract was kept in desiccator for further use.

Phytochemical screening

Phytochemical examinations of all the extracts were carried out to determine the presence of metabolites such as alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, according to standard methods.[11] Any visible change of color or precipitate formation was used as an indicative for the presence (+) or absence (–) of individual metabolites.

In vitro antioxidant assay

In vitro antioxidant screening of the extracts will be carried out in phased manner by different assays like.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

The free radical scavenging assay of various extracts was measured using a modified DPPH assay method[12] was used to measure the free radical scavenging assay of various extracts. Aliquots of 0.3 mL of various concentrations (50–250 µg/mL) of fraction were mixed with a solution of 0.2 mmol/L DPPH in methanol (2.7 mL). The mixture was vigorously mixed. Following incubation at room temperature for 15 mins in the dark, absorbance value was recorded at 517 nm using UV-Spectrophotometer.

The percentage of radical scavenging activity was determined using the following formula:

$$\text{Radical scavenging activity \%} = \{[AC - AS]/AC\}*100$$

Where *A*C was the absorbance of DPPH without sample and *A*S was the absorbance of the DPPH with sample.

2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

Free radical scavenging activity of various extracts were determined by ABTS radical cation decolorization assay[13] dark at room temperature for 12-16 h before use produced the ABTS·+ cation radical. An absorbance of 0.700 at 734 nm was obtained after diluting the ABTS·+ solution with methanol. After this, 5 µl of extracts were added to 3.995 ml of diluted ABTS·+ solution and the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out in triplicate. Percent inhibition of absorbance at 734 nm was calculated using the formula,

$$ABTS^{+} \text{ scavenging effect (\%)} = \{[AC - AS]/AC\} *100$$

Where *A*C was the absorbance of ABTS without sample and *A*S was the absorbance of the ABTS with sample.

Statistical analysis

Antioxidant activity results were expressed as mean ± standard error of the mean using Graph Pad prism software version 6.01 (Graph Pad software, San Diego, USA).

RESULTS

The extraction yield of SE was maximum observed i.e. 65% followed by SxE 43% and least in ME 22% w/w. The extracts were then kept in a desiccator to remove moisture and finally kept in a refrigerator for further

Table 1: Phytochemical Screening of *Syzygium cumini* Seeds Extracts.

S. No	Detection Test	SxE	ME	SE
	Alkaloids	++	+	+++
	Carbohydrates	+	+	+
	Glycosides	+	+	++
	Terpenoids	+	++	++
	Phenols	+	+	++
	Flavonoids	++	+	+++
	Fats	-	-	-
	Proteins	-	-	-
	Tannins	++	+	+
	Saponins	+	+	+

(-) Absent; (+) Present in a negligible quantity; (++) Present in moderate quantity; (+++): Present in a considerable quantity.

use. The preliminary phytochemical screening of all the extracts were tested with the standard procedure. Among all the extraction technique the SE extract showed maximum presence of the flavonoids, alkaloids and glycosides whereas absence of protein and fatty phytoconstituents depicted in Table 1.

All the extracts were evaluated for their antioxidant activity by using different antioxidant assays. The IC_{50} value for DPPH assay was lowest for MS with a value of 40 ± 8.11 µg/mL. The respective IC_{50} values for other two extracts i.e., SxE and SE were 40 ± 8.23 µg/mL and 69 ± 7.19 µg/mL respectively. The results were compared with the reference sample Naringenin which showed an IC_{50} value of 88.47 ± 6.41 µg/mL. The IC_{50} values in ABTS assay were 34 ± 5.31 µg/mL, 50 ± 7.95 µg/mL and 87 ± 8.19 µg/mL for ME, SxE and ME respectively. The IC_{50} value for the reference Naringenin was found to be 93 ± 8.11 µg/mL.

DISCUSSION

For the isolation of phytochemicals from the natural products there are various processes like milling, grinding, homogenization and extraction. Among all of these processes, extraction is one of the important process for separating phytochemicals from natural products.[14] The extraction effectiveness is affected by the chemical nature chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances.[15] Our study revealed that the ultrasound microwave assisted solvent extraction yield maximum followed by soxhlet extraction and least in maceration extraction. It is important to depict the chemical nature of plant materials when their pharmacological activities are evaluated. Phytochemical investigation of different extraction techniques of *Syzygium cumini* extracts revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics and glycosides as secondary metabolites. These secondary metabolites are reported to have many pharmacological properties, so this species is expected to have many medicinal uses.[16] The antioxidant assays which were aimed at assessing the potential of *Syzygium cumini* seeds extracts obtained from various extraction techniques in prevention of various diseases caused due to oxidative stress, gave variable results. The antioxidant potential of different was evaluated by radical scavenging capacity using UV–visible spectrophotometer. DPPH is a stable free radical. Antioxidants present in the natural products on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralising free radical character.[17] During the assay the colour of the reaction mixture changes from purple to yellow and its absorbance at wavelength 517 nm decreases. ABTS radical cation was produced in the stable form using potassium persulphate. After getting the stable absorbance, the antioxidant plant extract is added to the reaction medium and the antioxidant power was measured by studying decolorization.[18]

CONCLUSION

The current study provides the valuable evidence about the various extraction techniques, chemical composition and antioxidant properties. Different extraction techniques such as SxE, ME and SE were compared for *Syzygium cumini* seeds extraction on the basis of the extractive yields, antioxidant activities and extract composition. In conclusion, the antioxidants compounds present in *Syzygium cumini* have various beneficial effects towards the human health. Ultrasound microwave assisted solvent extraction is able to extract out phenolic compounds in high concentrations than other extraction techniques. Phenols are very important plant constituents; they show high scavenging ability of free radicals due to their hydroxyl group. Therefore, the phenolic content of plants may contribute directly to their antioxidant action.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay; **ABTS:** 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid assay.

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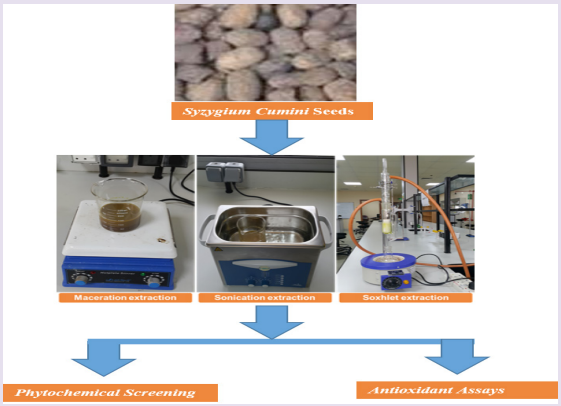
GRAPHICAL ABSTRACT



SUMMARY

- *Syzygium cumini* is used in complementary and traditional system of medicine in India.
- Phytochemical investigation of extracts revealed the presence of various secondary metabolites.
- Ultrasound microwave assisted solvent extraction is able to extract out phenolic compounds in high concentrations than other extraction techniques.
- *Syzygium cumini* also has antioxidant activity

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