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Quality assessment of *Syzygium aromaticum*: A pharmacognostic and phytochemical approach

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Abstract
Since from ancient time human beings are taking help of plants as a medicine, although most of these applications are anarchic. The worldwide herbal resources have a great potential as drug and are of great commercial importance. This use of herbal product has also given rise to various forms of abuse and adulteration of the products leading to patient's dissatisfaction and in some instances fatal consequences. They are very often obtained and processed without any scientific evaluation and launched onto the market without any safety and toxicology studies. There is a need to development of good quality assurance and standardization of herbal medicines and products. On that basis, an attempt was made on well-known herbal drug *Syzygium aromaticum* flower buds by evaluation of pharmacognostic, phytochemical and toxicological parameters like heavy metals, aflatoxins, total microbial load and pesticide residues.

Keywords: Pharmacognostic evaluation, *Syzygium aromaticum*, standardization, quality control

Introduction
Majority of the users trust on herbal medicines for health care because the other treatment options available are costly and they are often thought to be more associated with serious side effects. Medicinal plants are defined as a group of plants that possess some distinct properties that qualify them as articles of drugs and therapeutic agents. Medicinal plants contain some organic compounds having definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [1]. These Chemical constituents having therapeutic activity found in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases. Most of the medicines today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional and alternative systems of medicine. Spices play a very important role as a medicine in healthcare system. Spices like clove, black pepper, turmeric, nutmeg and cinnamon have been used for centuries as food preservatives, taste modifier and as medicinal plants mainly due to its antioxidant and antimicrobial activities [2]. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to its active use in food & pharmaceutical industry standing out among the other spices. Essential oil compounds are fat soluble thus possess the ability to permeate the membranes of the skin before being captured by the microcirculation and drained into the systemic circulation which reaches all targets organs [3]. Clove (*Syzygium aromaticum*) is one of the most commonly used spices in Indian kitchens. Cloves are the aromatic dried flower buds of a tree *Syzygium aromaticum*, native to the Maluku islands in Indonesia and used as a spice in cookeries all over the world [4]. Clove oil has germicidal properties and is frequently used in the dental care due to its germicidal properties. It has been shown to be a potent chemo preventive agent, used by the traditional Ayurvedic healers of India since prehistoric times to treat respiratory and digestive ailments [5].

Whole and ground cloves are used to enhance the flavor of meat and rice dishes and used widely in curry powders and masalas. They are highly valued in medicine as a carminative and stimulant and are said to be a natural anthelmintic [6]. Oil of clove is used extensively for flavoring all kinds of food products, such as meats, sausages, baked goods, confectionery, candies, table sauces, pickles, etc. It is used in medicine for its antibacterial, antiseptic and antibiotic properties [7, 8]. It has also been successfully used for asthma and various allergic disorders by oral administration. Sesquiterpenes, found in clove were also investigated as potential anti-carcinogenic agents. The oil has many industrial applications and is used extensively in perfumes, soaps and as a clearing agent in histological work.

In addition, the cloves are anti-mutagenic, anti-inflammatory, antioxidant, antiviral, anti-thrombotic and anti-parasitic [9, 10]. Clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenyl acetate (15%) and β-caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid [11, 12]. The major pharmacological activities of clove are antifungal, antibacterial, carminative, antiseptic, anti-emetic, anti-oxidant, analgesic and insecticidal properties alongside as a flavouring agent in culinary. Some other activities are aphrodisiac, mosquito repellant, insecticidal and antipyretic [13]. The process of standardization can be achieved by stepwise pharmacognostic and Phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. The present study includes identification, Characterization and standardization of clove flower buds [14].

Materials and Methods

The existing work relates to standardization of clove flower buds, as per WHO guidelines.

Collection of Sample

For the current study, clove flower buds (*Syzygium aromaticum*) were procured from S. G. Phytopharma Pvt. Ltd., Kolhapur. Their identity and Authentication was confirmed by Department of Pharmacognosy Marathwada Mitra Mandal's College of Pharmacy Pune by correlating their morphological and microscopical characters with those given in literatures. The remaining flower bud samples were dried in shade. Coarse powder (60 #) of dried flower buds was stored for their microscopical study, physicochemical evaluation and phytochemical investigations.

Micromorphology

The organoleptic characters of the fresh flower buds and dried flower bud powder like colour, odour, taste and the macroscopic characters viz, size, shape were evaluated as per standard WHO guidelines [15].

Cytomorphology

The transverse sections of flower bud were cleared, mounted in glycerin water and observed under Digital microscope (MOTIC-B1). Microscopy of dried flower buds powder was studied for evaluation of various parts present in given drug powder; the detail cytomorphological characters were observed and reported [16, 17].

Quantitative Microscopy

Quantitative microscopy of flower bud sample was performed as per WHO guidelines to determine various cellular dimensions.

Physicochemical Evaluation

Physicochemical parameters such as colour, smell, ash value viz., total ash, acid insoluble ash was evaluated. The water soluble and alcohol soluble extractive values were determined according to standard official procedure [18, 19].

Preliminary Phytochemical Investigations

The qualitative chemical tests carried out for the identification of the nature of phyto-constituents present in the powered crude drug [16].

Determination of Pesticide, Aflatoxin and Microbial load

Determination of Pesticide, Aflatoxin and Microbial load was done as per WHO guidelines [20, 21].

Heavy metal analysis

Heavy metal analysis was done by Atomic Absorption Spectroscopy recommended by WHO so as to determine heavy metal contamination [22].

Result and Discussion

Macromorphological Description

Cloves are the immature unopened flower buds. The flower bud has a spherical head and a sub-cylindrical hypanthium tapering at the lower end. Calyx is polysepalous having four hard and thick sepals. Corolla is polypetalous having four petals imbricate, encloses the stamens and forms the head of the bud.

Table 1: Macromorphological Description

Sr. No.	Characters	Observation
	Organoleptic Characters	
1	Color	Crimson to Dark Brown
2	Odor	Aromatic and strong
3	Taste	Pungent, aromatic
	Quantitative Macromorphology	
4	Size	10-15 mm in length, 3.5mm in width, 2 mm thick
	Macroscopical features	
5	Shape	Hypanthim is surrounded with 4 thick acute divergent sepals surrounded by dome shaped corolla. The corolla consists of unexpanded membranous petals with several stamens & single stiff prominent style.

Cytomorphological Description

Fig 2 & 3 reveals the transverse section of the flower bud through columella shows the presence very thick cuticle. Cortex region comprises of three zones, upper zone contains radially arranged parenchymatous cells with two or three layers of big, ellipsoidal oil glands. Middle zone contains a ring of bicollateral vascular bundles. Isolated pericyclic fibers are present around the vascular bundle. Lower zone of parenchyma shows the presence of air spaces (Aerenchyma). The parenchyamatous cells rich in calcium oxalate clusters. 25-30 small vascular bundles are present at periphery.

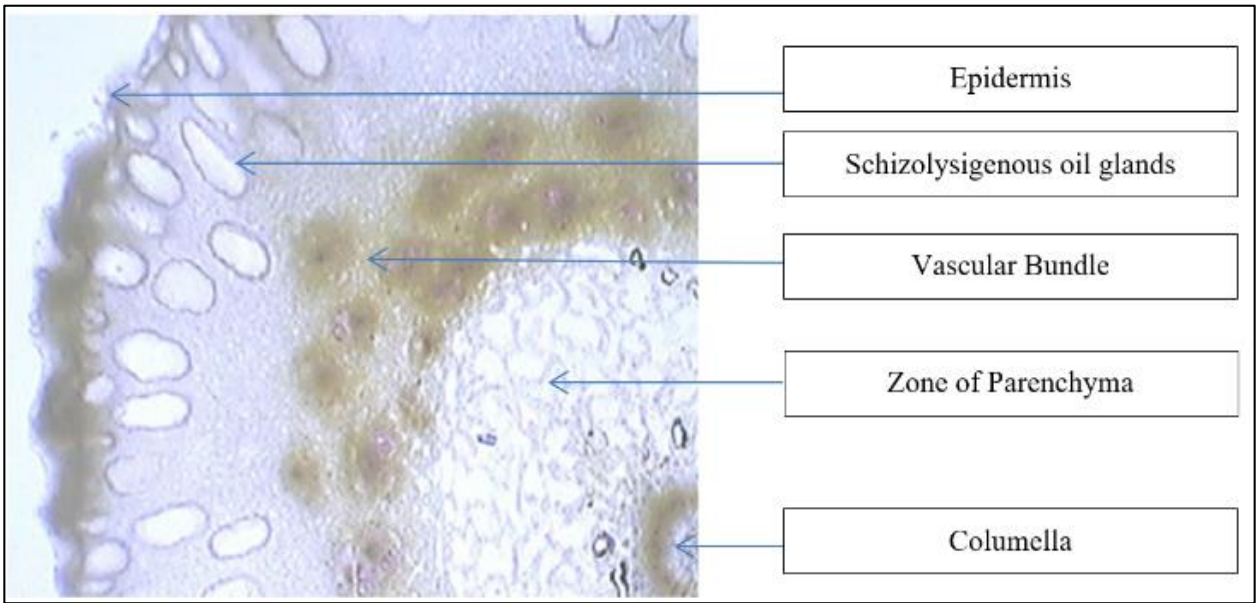
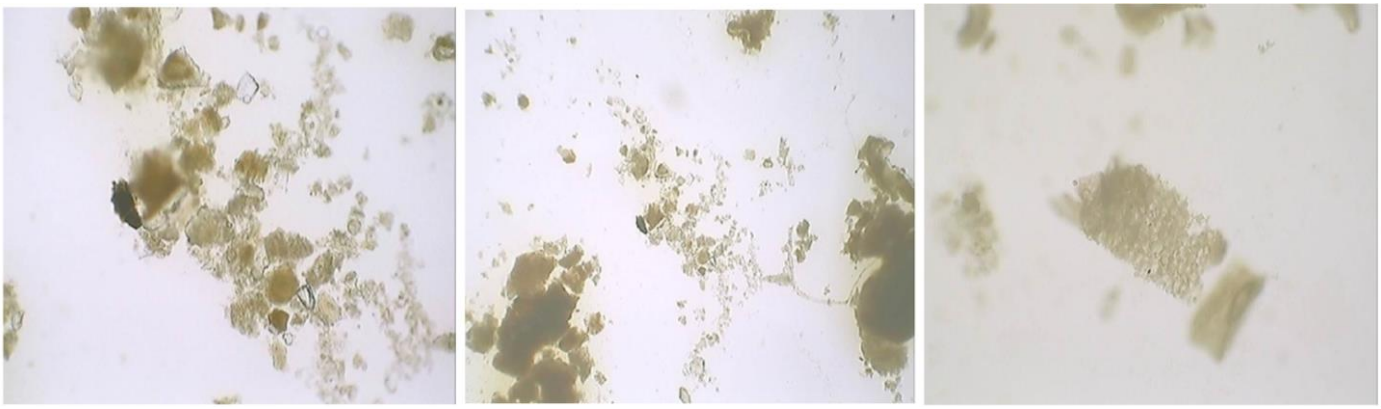


Fig 1: T. S. Clove bud



Sclerenchymatous fibers Pollen grains Oil glands

Fig 2: Microscopic Characteristics of Powdered drug

Physicochemical Parameters

The result of the physicochemical constants of clove flower buds is within the limit which is mentioned in Table 2; this signifies that the quality and purity of raw material was good enough. The results of Ash values signify the content of inorganic material mainly the content of metallic salts and silica present in the raw material, the values are 5.6 ± 0.52% for total ash and 0.8 ± 1.00% for acid soluble ash value; which are within fairly wide limit. Extractive values are used to determine the amount of active constituents in given amount of medicinal plants which is qualitative as well as quantitative estimation of phytoconstituents. Extractive values act as preliminary information about the drug; the water soluble extractive value found to be 21.60 ± 3.33% while the alcohol soluble extractive value was found to be 10.42 ± 2.88% which signify the nature of the phytoconstituents present in plant. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important, equally important is the rate at which the moisture is removed and the condition under which it is removed. Thus the determination of moisture content also provide the method of preparation of drug; and it is observed that the moisture content of the drug was found to be 6.96 ± 0.16%w/w which signify that the drug is properly dried and properly stored. The results of all physicochemical parameters were in table 2

Table 2: Physicochemical Parameters

Sr. No.	Parameters	Standard
1	Foreign organic matter (% w/w)	1.8%
	Ash Values	
2	Total ash (% w/w)	5.6%
3	Acid insoluble ash (% w/w)	0.8%
	Extractive Values	
4	Alcohol soluble extractive value (% w/w)	10.42%
5	Water soluble extractive value (% w/w)	21.60%
	Physical Constants	
6	Moisture content (IR balance) (% w/w)	6.96%

Preliminary Phytochemical Screening

The preliminary phytochemical investigations of powdered clove buds were performed which shows the presence of tannins, phlobatannins, phenolic compounds, terpenoids type of major secondary metabolites which revealed their potent therapeutic activity. The results of the screening were express in Table 3.

Table 3: Preliminary Phytochemical Screening

Parameters	Distilled water extract	Methanolic extract
Tannins	Present	Present
Phlobatannins	Present	Present
Phenolic compounds	Present	Present
Reducing sugars	Present	Present
Terpenoids	Present	Present

Determination of Pesticide, Aflatoxin and Microbial load

The contents of heavy metals namely lead, mercury, cadmium and arsenic are found to be within the permissible limit for the *Syzygium aromaticum*, indicating that the flower buds are safe to utilize as drugs. The report for analysis of aflatoxins in the *Syzygium aromaticum* shows that the aflatoxins B1, B2, G1 and G2 were below the detecting level revealing that they are free from toxins and are safe for internal use. Further the studies indicated that the absence of these aflatoxins would help to increase in shelf life of the raw drug. The Pesticide residues are analysed and none of the mentioned pesticides is detected in the flower buds, indicating that they are safe for their usage as drugs (Table 4). Analysis for the microbial load for the *Syzygium aromaticum* is found to be within the limit of WHO guidelines, indicating that they are free from pathogens and can be used as drugs (Table 5).

Table 4: Pesticide Residue

Testing Parameter	Method (WHO)	Testing Parameter	Method (WHO)	Testing Parameter	Method (WHO)
Dichlorvos	Complies	α-Endosulfan	Complies	Trans-Permethrin	Complies
Parathion-methyl	Complies	Dieldrin	Complies	Cypermethrin	Complies
Chlorpyrifus-methyl	Complies	p,p'-DDE	Complies	Fenvalerate	Complies
Pirimiphos-methyl	Complies	Endrin	Complies	Delta Methrin	Complies
Malathion	Complies	β-Endosulfan	Complies	o,p'-DDT,HCH	Complies
Parathion	Complies	o,p'-DDT	Complies	o,p'-DDT,HCL	Complies
Chlophyrifos	Complies	Carbophenothion	Complies	PCB (Polychlorinated bophenyl)	Complies
Ethion	Complies	p,p'-DDT	Complies	o,p'-DDE	Complies
Carbophenothion	Complies	Cis-Permethrin	Complies	ε- Hexachlorocylophenane	Complies
Lindane	Complies	α-Hexachlorocylohenane	Complies	Heptachlor	Complies
Gamma-hexachlorocylohenane	Complies	Hexachlorocylohenane	Complies	β- Hexachlorocylophenane	Complies
Aldrin	Complies	Cis-Heptachlorepoxide	Complies		

Aflatoxins Analysis: Total Aflatoxins (B1, B2, G1, G2) ≤ 5µ**g/kg-**

Table 5: Microbial Analysis

Sr. No.	Test	Result
Microbial Limit tests		
1	Test for *E. coli*	Absent
2	Test for *Salmonella enterica*	Absent
3	Test for *Ps.aeruginosa*	Absent
4	Test for *S.aureus*	Absent
Total Aerobic microbial count		
1	Total bacterial count	70 cfu/gm
2	Total fungal count	< 10cfu/gm
3	Total yeast & mould count	< 10cfu/gm

Table 6: Heavy Metal Analysis

Sr. No.	Haevy metals	Result	Maximun Limit
1	Lead	0.694ppm	10ppm
2	Cadmium	Nil	0.3ppm
3	Mercury	Nil	1.0ppm
4	Arsenic	Nil	10ppm

Conclusion:

The flower buds of *Syzygium aromaticum* exhibit a set of diagnostic characters, which will help to identify the drug in dried condition. It has been concluded from this study that assessment of heavy metals and pesticides residue, aflatoxin and total microbial load is highly indispensable for raw drugs or plant parts used for the preparation of single and compound formulation drugs. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs; it is clear that these herbal medicines will offer a platform for further research. Hence the periodic assessment is important for quality assurance and safer use of herbal drugs.

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