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DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR IDENTIFICATION OF PHYTOCHEMICALS IN SELECTED HERBAL DRUGS

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ABSTRACT

The present work attempted to identify and standardize the different phytochemical by modern analytical techniques and to summarize the UV, TLC, FTIR characters of the plants; *Eugenia caryophyllus* (clove), *Acorus Calamus* (vekhand), *Myristica fragrans* (Nutmeg) and *Citrus aurantium*(Orange peel) which ultimately results in compilation of analytical data for these crude drugs which are very much used in herbal cosmetics. The methanolic extracts of Clove, Acorus, Nutmeg, Orange peel were prepared by ultrasonic bath extraction method and labeled as DSC, DSA, DSN and DSO respectively for further studies. Preliminary pytochemical screening of extracts were also performed. Asarones were detected in Acorus extract by subjecting the TLC to UV light and observing blue fluorescence. Volatile oil was isolated from clove buds by using Clevenger's apparatus which was subjected for the FTIR analysis, which indicates the peak for presence of phenol compound. For nutmeg and orange peel samples the presence of myristicine, elemicine in nutmeg extract was confirmed by Rf values.

INTRODUCTION: The present investigation deals with the studies of important techniques for identification of main phytochemical present in crude drugs which can be helpful in authenticating the plant material and also establish the data for quality control of crude drugs.

For present work we have selected four crude drugs which belong to the category of volatile oil [1] and which are regularly used in herbal cosmetic industry [2]. This obtained data is compared with standard values or the data from the literature which gives the confirmation about the identified phytochemical. Clove oil [1] (*Eugenia caryophyllus*) contains Eugenol (**Fig. 1**) (70-90%) [3], eugenol acetate, caryophyllenes and small quantities of esters, ketones, and alcohols. Oil of clove[4] is colorless to pale yellow in color.

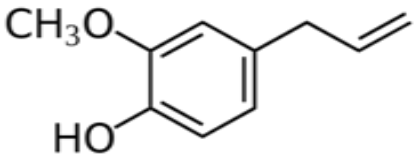

FIG. 1: STRUCTURE OF EUGENOL

It becomes thick and darker in color on storage. Traditionally clove is used as dental analgesic, carminative, stimulant, flavoring agent, an antiseptic [5], in the preparation of cigarettes. The oil is used in perfumery and also in the manufacture of vanillin.

Vekhand (*Acorus Calamus*) contains asaraldehyde, asarone (**Fig. 2**) and eugenol also a bitter amorphous principle known as acorine [6, 7]. Methanolic extract of acorus shows antidiarrheal action neuroprotective properties. Alcoholic extract have sedative and

analgesic activity, also used as traditional remedy for pain, convulsion, inflammation and ulcer [8].

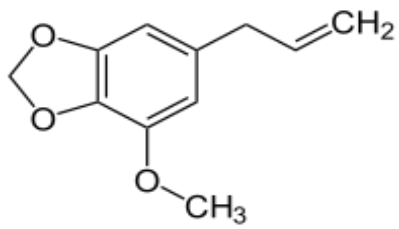

FIG. 2: STRUCTURE OF ASARONE

Nutmeg (*Myristica fragrans*) contains a-pinene, camphene, b-pinene, sabinene, myrcene, a-phellandrene, a-terpinene, limonene, 1, 8-cineole, y-terpinene, linalool, terpinen-4-ol, safrole, methyl eugenol and myristicin [9]. Myristicin is a weak inhibitor of monoamine oxidase. The fat (known as banda soap) and volatile oil of nutmeg is used in treatment of rheumatism anti-diarrheal activity, antioxidant effect and antibacterial activity against *E-coli* in vitro.

Simpson *et al* performed the analytical study and revealed a plot from a GC/MS of the volatile oil (**Fig. 3**) from the nutmeg seed which is given below and the numbered peaks are identified as:

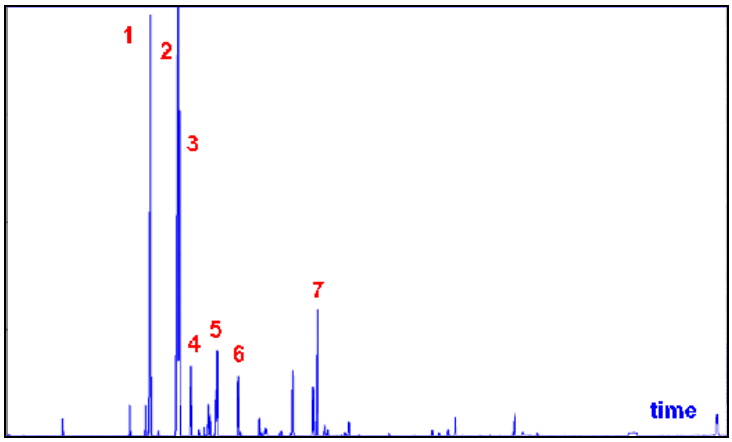

FIG. 3: GC/MS OF THE NUTMEG VOLATILE OIL

(2902) α-pinene MOL or MS; sabinene PDB or MS; (2903) β-pinene PDB or MS; myrcene PDB or MS; (2633) limonene MOL or MS; α-terpinene PDB; (2248) terpinen-4-ol PDB or MS among the major ingredients; others are α-phellandrene, p-cymene, linalool, and α-terpineol. A recent study on nutmeg oil from St Catherine, Jamaica and other West Indian nutmeg oils revealed that except p-cymene and α-terpineol the Jamaica nutmeg oil was found to contain all compounds in high concentration as compared to Grenada and Indonesian variety [10, 11].

The orange peel (*Citrus aurantium*) contains 2.5% of volatile oil; it also contains compounds like hesperidin, neohesperidin, iso-hseperidin, vitamin C and pectin[9]. The bitter substances are the glycosides aurantimarin and aurantimarin acid. Volatile oil contains 90% of limonene and small % of aldehyde, chiefly citral and citronellal. It is used in chemical synthesis as a precursor to carvone and as a solvent in cleaning products. Limonene causes sedation and peripheral analgesia. The hesperdin shows antioxidant effect according to *in vitro* studies [12].

Hesperidin reduced cholesterol [13] and blood pressure [14] in rats. In a mouse study, large doses of the glucoside hesperidin decreased bone density loss[15] another animal study showed protective effects against sepsis [16]. Identification of hesperidin was carried out on HPLC system by using the mobile phase consisted of a mixture of acetonitrile water and methanol (20:79, 6.0,4 V/V) [17] as depicted in **fig. 4**.

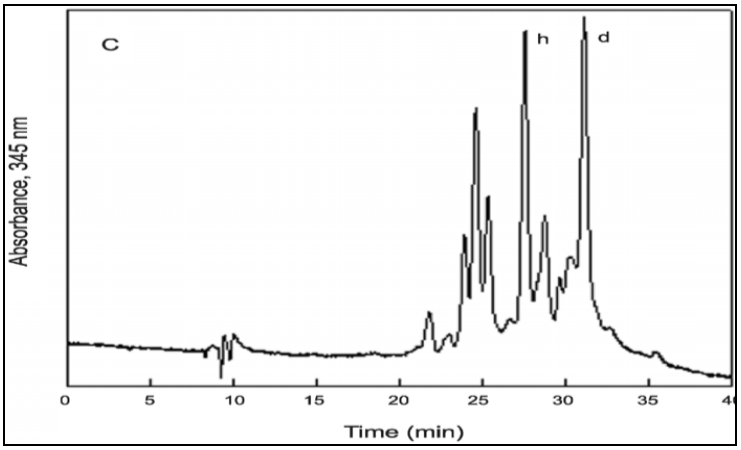

FIG. 4: HPLC PATTERN OF HESPERIDIN

MATERIALS AND METHODS:

Plant material: The crude drugs; Clove (*Eugenia caryophyllus*),Vekhand (*Acorus Calamus* Linn.), Nutmeg (*Myristica fragrans* Houtt.), Orange peel (*Citrus aurantium* Linn.) were collected from Pune, Maharashtra in the month of June, 2010, properly dried and stored in air tight containers. It was authenticated at Agarkar Research Institute, Pune, India.

Preparation of extracts: For extraction [18] all crude drugs were pulverized using a mixer. The coarse powder (50 gm) was subjected to successive extraction with methanol (200 ml) by maceration in a conical flask

with stopper and periodic ultrasonification. The marc obtained was further subjected to re-extraction using same solvent. All the extracts obtained were concentrated using laboratory grade hot plate and further subjected for the chromatographic and spectroscopic analysis. The clove oil was extracted from 100gm of clove bud powder and then the oil was packed in air-tight container for further study.

Experimental work:

Phytochemical investigation by chemical tests: All the extracts were subjected to phytochemical investigation as per official book[19] for detection of various phytochemicals. All extract were subjected to solubility tests as well as chemical tests for confirmation of active constituents, as given below;

Clove oil + alcohol + ferric chloride (5%) solution were mixed it shows the blue coloration.

Powdered nutmeg on micro-sublimation produces sublimates of colourless crystals of myristicin.

Orange peel powder + 7% potassium hydroxide solution it shows yellow coloration which confirms presence of orange peel.

Presence of asarones in Acorus is confirmed by chromatography method.

Spectroscopic measurement [20]: Accurately weighed quantity of drug extract 10 mg was dissolved in methanol by using volumetric flask and volume was adjusted to 10 ml by using solvent methanol so as to get 1000 ug/ml stock solution. Stock solution was further diluted to produce 10, 30, 60, 90, 120, ug/ml as final concentrations. For these dilutions absorbance was measured between 200 to 400 nm by using double beam Spectrophotometer, (Jasco: V-630) and reported here.

Chromatographic analysis [21]: Silica gel slurry was prepared using water and slides were prepared by pouring the slurry on glass slides. These plates were kept in oven for 10 minute for activation. Saturated solution of drug extract was prepared in methanol. Developing chamber was kept ready with proposed solvent system for saturation [22]. The spotting was done in such a way that approximately 1 mm-sized spot of the solution is seen.

The plate was kept slightly tilt in solvent system to run the solvent system $3/4^{th}$ of the plate. The spots made visible by using iodine chamber and further photographs were recorded and Rf was calculated.

RESULTS AND DISCUSSION: Ultrasonic bath extraction was found to be most efficient way for getting maximum yield. All extracts (**Fig. 5**) were soluble in methanol, benzene and chloroform. The characterization of extract is depicted in **Table 1.**

FIG. 5: EXTRACTS STORED IN FREEZE

TABLE.1: CHARACTERS OF ALL EXTRACTS AND OIL ALONG WITH % YIELD

Drug	Color of extract	Phytochemicals present	Confirmatory test	% Yield
DSC	Dark brown, sticky	Tannins, terpenoids,	Eugenol, Tannins	18
DSC-oil	Faint yellow, turbid	Volatile oil, terpenoids	Eugenol	3.5
DSA	Brown, shiny, smooth	Carbohydrates, starch	Asarones	0.6
DSN	Yellowish, flex, smooth	Fats, terpenoids, sugars, proteins	Myristicin	26
DSO	Brown and woody, sticky	Vit. C, carbohydrates, tannins	Tannins, pectin	12

Thin layer chromatography for each extract was carried out by using different solvent systems and developed the proper solvent system combination for exact visualization of spot (**fig. 6 to fig. 9**). The UV analysis of all crude drugs has shown different values of λ_{max} which depicted in **fig. 10.**

The photographs of TLC and details of Rf, solvent system and their ratio is as depicted in **Table 2** and name of the compound was found out as per detail literature survey.

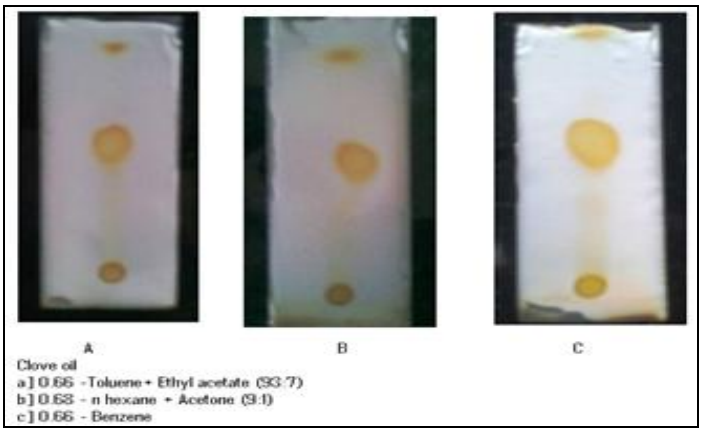

FIG. 6: TLC FOR CLOVE OIL

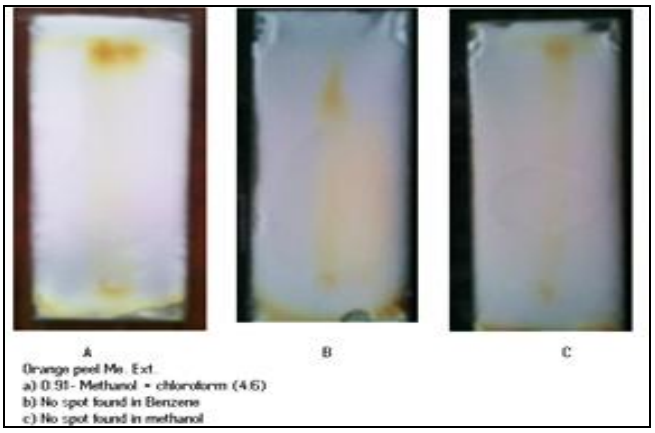

FIG. 8: TLC FOR NUTMEG

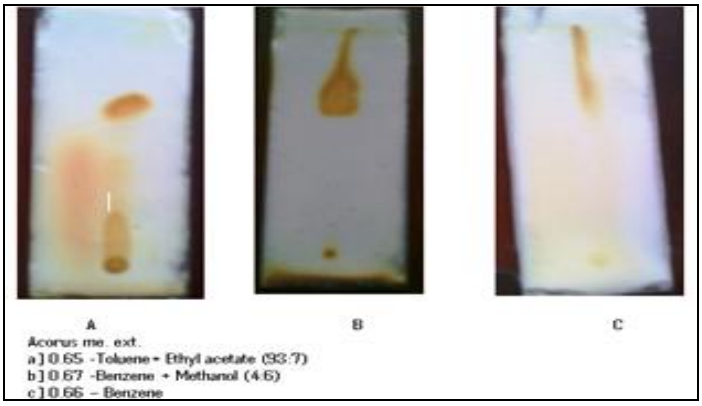

FIG. 7: TLC FOR ACORUS

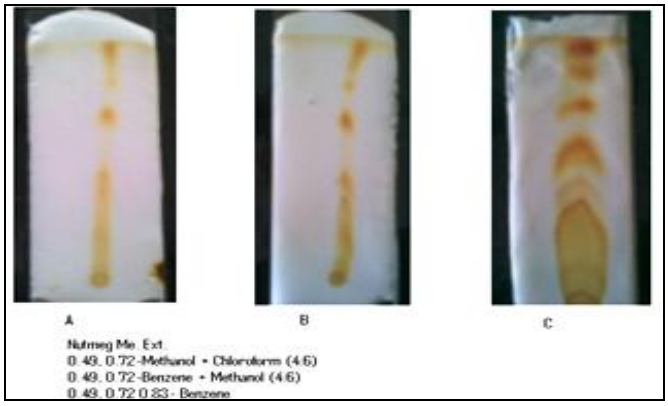

FIG. 9: TLC FOR ORANGE PEEL

TABLE.2: TLC AND UV ANALYSIS OF ALL EXTRACTS AND OIL

Drug	λ_{max} (UV analysis) *	Solvent system & its ratio (TLC) **	Rf	Name of compound
DSC	279 nm	Toluene + Ethyl acetate (93:7)	0.66	Eugenol
DSC-oil	279 nm	Toluene + Ethyl acetate (93:7)	0.68	Eugenol
DSA	299 nm	Benzene + Methanol (4:6)	0.67	Not known
		Benzene	0.81	Myristicin,
DSN	274 nm	Methanol+ Chloroform (4:6)	0.72	Elimicin
		Methanol+ Chloroform (4:6)	0.49	Safrol
DSO	278 nm	Methanol + Chloroform (4:6)	0.61	Not known, may be hesperidin

* - Two reading were taken for all extracts; * * - Three reading were taken for all extracts

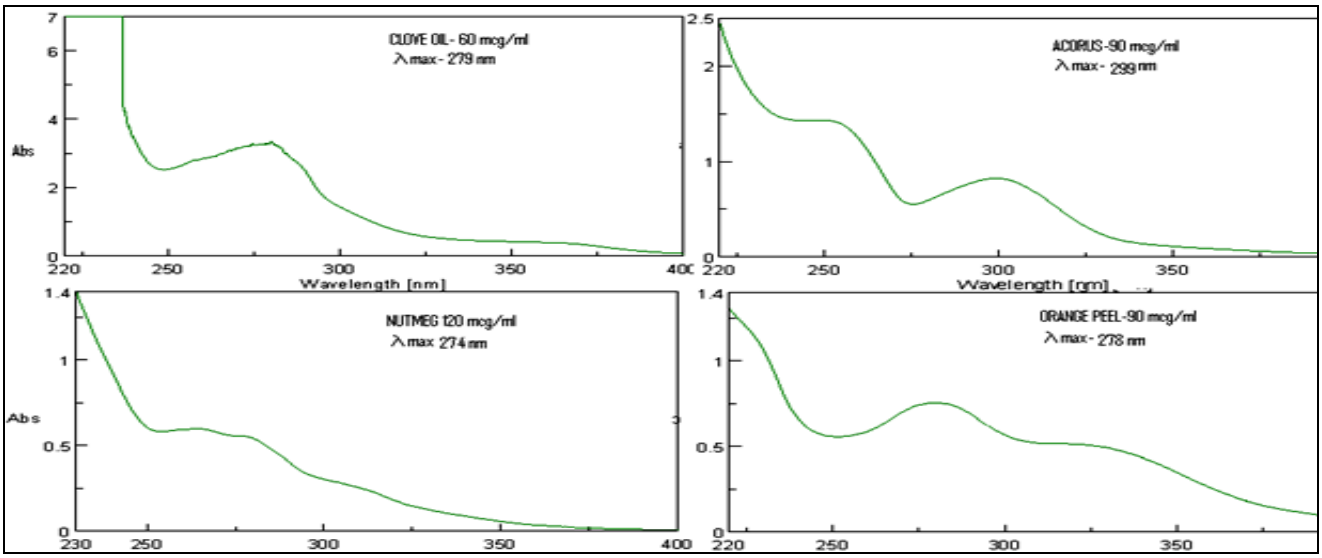

FIG. 10: UV SPECTRUM OF DSC, DSA, DSN AND DSO

Clove oil and Acorus methanolic extract were subjected to spectral analysis by using Fourier transform infrared Spectrophotometer; Jasco make. & the spectra obtained are depicted in **fig. 11** and **fig. 12**.

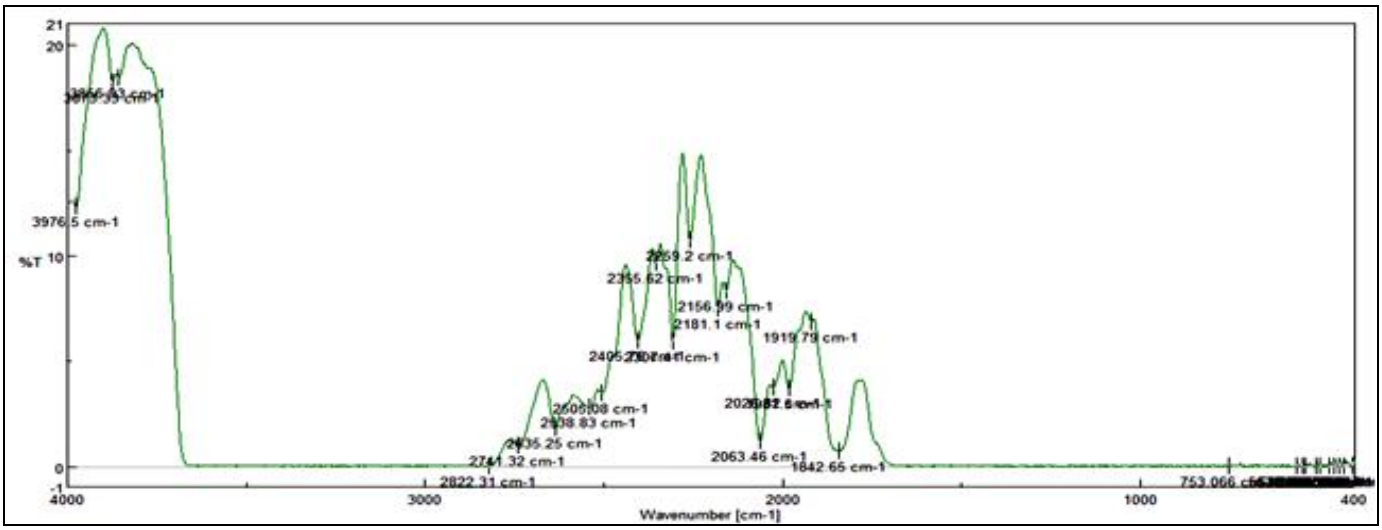

FIG. 10: FT/IR SPECTRA OF CLOVE OIL

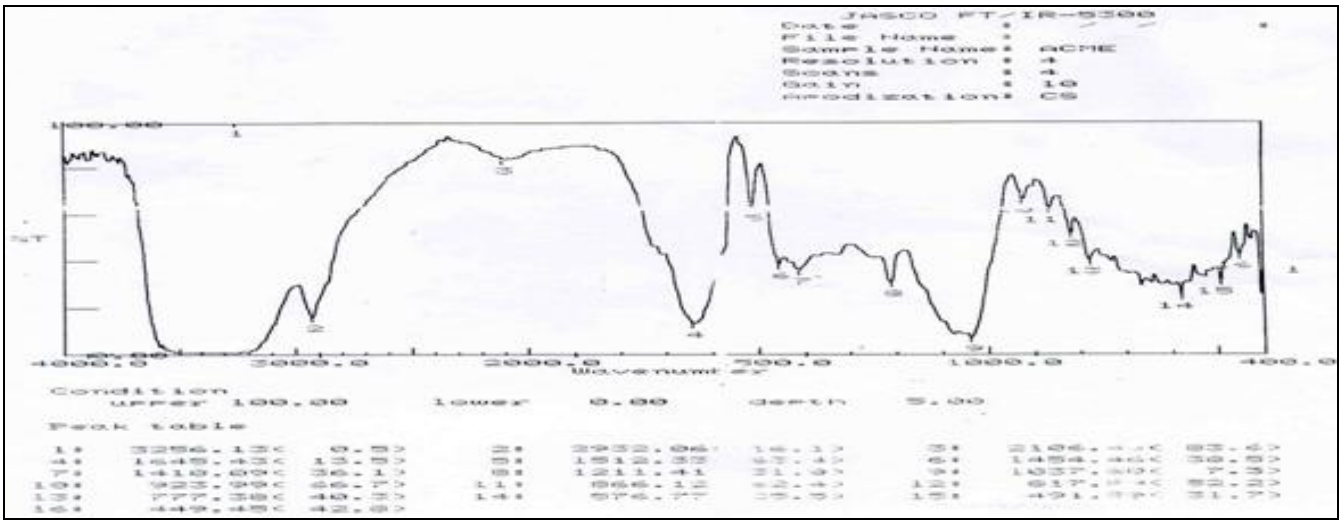

FIG. 11: FT/IR SPECTRA OF DSA

The IR data shows presence of following groups for clove oil;

2822.31 cm^{-1} -OH, 2741.32 cm^{-1} -CHO, 2635.25 cm^{-1} –C=C, 2405.02 cm^{-1} –C=C, 1882.6 cm^{-1} –C=O, 1842.65 cm^{-1} – O- .

The IR data shows presence of following groups for Acorus methanolic extract;

3256.13 cm^{-1} -OH, 1645.43 cm^{-1} –C=C ,1410.09 cm^{-1} –N-H, 923.99 cm^{-1} –C-C, 1512.33 cm^{-1} C_6H_6, 1211.41 cm^{-1} – C-O, 2106.43 –alkynes.

From above data one can confirm the presence of eugenol in clove by phytochemical analysis and chemical test which is further reconfirmed by λ max value, Rf value and FTIR.

In nutmeg, the presence of myristicine was confirmed by specific chemical test and presence of myristicine, elemicine and safrol was confirmed by TLC studies. For nutmeg oil one can confirm the presence of α-pinene, sabinene, β-pinene, myrcene, limonene, α-terpinene and terpinen-4-ol by studying the GC/MS peaks given here nutmeg also shows presence of high quantity of fatty substances.

Asarones show blue fluorescence by exposing TLC to UV light, as well as by phytochemical analysis which is reconfirmed by λ max value and Rf value. All extracts has given positive tests for presence of volatile oils and tannins.

CONCLUSION: In conclusion, the present study on clove, acorus, nutmeg and orange peel may be useful to supplement the analytical information with regard to their identification and can be useful as an authenticating parameter of standardization. In future we are planning to generate databank for a particular class of phytochemical which are currently used in herbal and cosmetic products.

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