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QUALITATIVE ASSESSMENT OF KAYATHIRUMENI - A SIDDHA MEDICATED OIL AS A REMEDY FOR ARTHRITIC PAIN

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Key words
Kayathirumeni, Varma oil, Thailam & chromatography

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ABSTRACT

Plan: *Kayathirumeni is an important formulation of Siddha system of medicine used to treat various human disorders. Such as myalgia, Arthritic pain etc.*
Preface: *The present study aimed to set quality parameters in physico-chemical, Chromatography, Microbiology and Heavy metals for Kayathirumeni.*
Methodology: *Assays for ash, extractive value, phytochemical profile, Heavy metal assay.*
Outcome: *The finding obtained from the study might be used as standard values for Kayathirumeni.*

1. INTRODUCTION

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety, and efficacy[1].

Nevertheless most of the Polyherbal formulation gaining importance due to their long historical clinical use and reliable therapeutic efficacy, traditional Indian medicine attract and increase global attention and many big pharmaceutical companies are using traditional Indian medicine as an excellent pool for discovering natural bioactive compounds.

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With the growing need for safer drugs, attention has been drawn to their quality, efficacy and standards of the traditional Indian medicine formulations[2]. Therefore the quality control has paid much importance not only the purity, quality and also stability of the products.

Siddha Medicine encompasses thirty two kinds of internal medicines and thirty two kinds of external medicines. Thailam or ennai are medicated oils listed under internal medicines with shelf life of one year according to Siddha literature[3]. Commonly the medicated oils are used for muscular pain and sprain as topical application. Certain oils are indicated for both internal and external. The preparation of thailam involves many crude drugs, herbal juice extracts, decoctions and various medicines as the base material to extract the active constituents from new drugs. The base material may be any oil like Coconut oil, Gingili oil, Castor oil, Neem oil etc., depending upon the disease as they are good vehicles.

Kayathirumeni is one of the important traditional poly herbal medicated oils used for various ailments. It plays major role in internal and external wound healing, sprain, fractures, head ache, sinusitis, giddiness, haemoptysis, and generalised tiredness. It regulates varmam energy flow in the body and balances three body humours vatham (air flow), pittam (heat) and silaethumam (phlegm) [4].

However, the characteristics of traditional Indian medicine are their systematise, multi-target and multichannel due to their complex chemical constituents. This needs to be studied and scientifically understood [5]. Literary search revealed that so far, no standardization work has been executed on Kayathirumeni oil. Hence, the available literature revealed that there is no standardization study has been done on Kayathirumeni. The aim of the present study is to evaluate the organoleptic, physico-chemical, phytochemical and chromatography standards of Kayathirumeni thailam (Medicated oil).

2. MATERIALS AND METHODS

Pharmacognostically pure and authentic ingredients were used for the preparation of medicated oil.

S.NO	*Tamil Name*	*Scientific name*	*Parts used*
1.	Kuppaimeni	*Acalypha indica*	Whole plant
2	Nal velai	*Gynandropsis pentaphylla*	Whole plant
3	Kayanthagarai	*Wedelia chinensis*	Whole plant
4	Sangankuppi	*Clerodendron inerme*	Leaves
5	Nathaichoori	*Spermacoce hispida*	Roots
6	Kovai	*Cephalandra indica*	Leaves
7	Eechuramooli	*Aristolochia indica*	Roots
8	Amirthavalli	*Tinospora cordifolia*	Leaves
9	Utthamani	*Daemia extensa*	Whole plant
10	Omavalli	*Anisochilus carnosus*	Leaves
11	Musumusukkai	*Mukia maderaspatana*	Whole plant
12	Vetrilai	*Piper betle*	Leaves
13	Kaarpogi	*Psoralea corylifolia*	Seeds
14	Karun jeeragam	*Nigella sativa*	Seed
15	Devadharam	*Cedrus deodara*	Heart wood
16	Vilamicham ver	*Plectranthus amboinicus*	Roots
17	Koshtam	*Costus speciosus*	Roots
18	Sandhanam	*Santalum album*	Heart wood

19	Jaathikkai	*Myristica fragrans*	Fruits & arils
20	Ramicham ver	*Vetiveria zizanioides*	Roots
21	Seeragam	*Cuminum cyminum*	Fruits
22	Chitrarathai	*Alpinia chinensis*	Rhizomes
23	Marul	*Sansieviera roxburghiana*	Leaves
24	Vellai kungiliyam	*Shorea robusta*	Gum
25	Purified thurusu	*Purified Blue Vitriol*	-
26	Purified thutham	*Zinc sulphate*	Metal
27	Thengai ennai	*Cocos nucifera*	Oil
28	Pachai karpuram	*Borneo camphor*	-

2.1. Preparation of medicated oil

All the fresh herbs were procured from Nager coil (Kanyakumari district, Tamil Nadu); crude raw drugs were procured from local traders and authenticated by a qualified botanist in our research centre. The purity and quality of the herbal raw materials were confirmed by the Pharmacognostical analytical method guided by Siddha Pharmacopoeia of India[6]. The oil was prepared at Arivan Siddha pharmacy in a batch size of 20 litres as per the literature and under supervision of a Siddha physician (*Vaidya*). There was no colouring agent, perfumes and antioxidant or stabilizers were added to the oil. The final batch were transferred into clean air tight pet containers and stored at dry place. The samples were taken for the further analysis (Fig.1).

2.2. Evaluation of physicochemical parameters:

Preliminary physicochemical parameters such as colour, odour, appearance, specific gravity, Refractive index, saponification value, peroxide value, acid value, Kries test and iodine value were determined in Kayathirumeni oil according to the standard techniques [7].

2.3. Determination of specific gravity

Fill the dry pycnometer with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 30^{O}C and hold for 30 min [7].

Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh ensuring that the temperature does not fall below 30 ºC.

2.4. Determination of acid value

Weigh 5 gm. of oil and transfer it into 250 ml conical flask. Add 50 ml of neutralized alcohol solution (25ml of alcohol and 25 ml diethyl ether) to the oil solution. Heat this mixture for 10 minutes by using the heater. Take the solution after 10 minutes and add 1 or 2 drops of phenolphthalein indicator. Titrate against the 0.1 M KOH solution from the burette. The appearance of pink colour indicates the end point (Page: 100)[7].

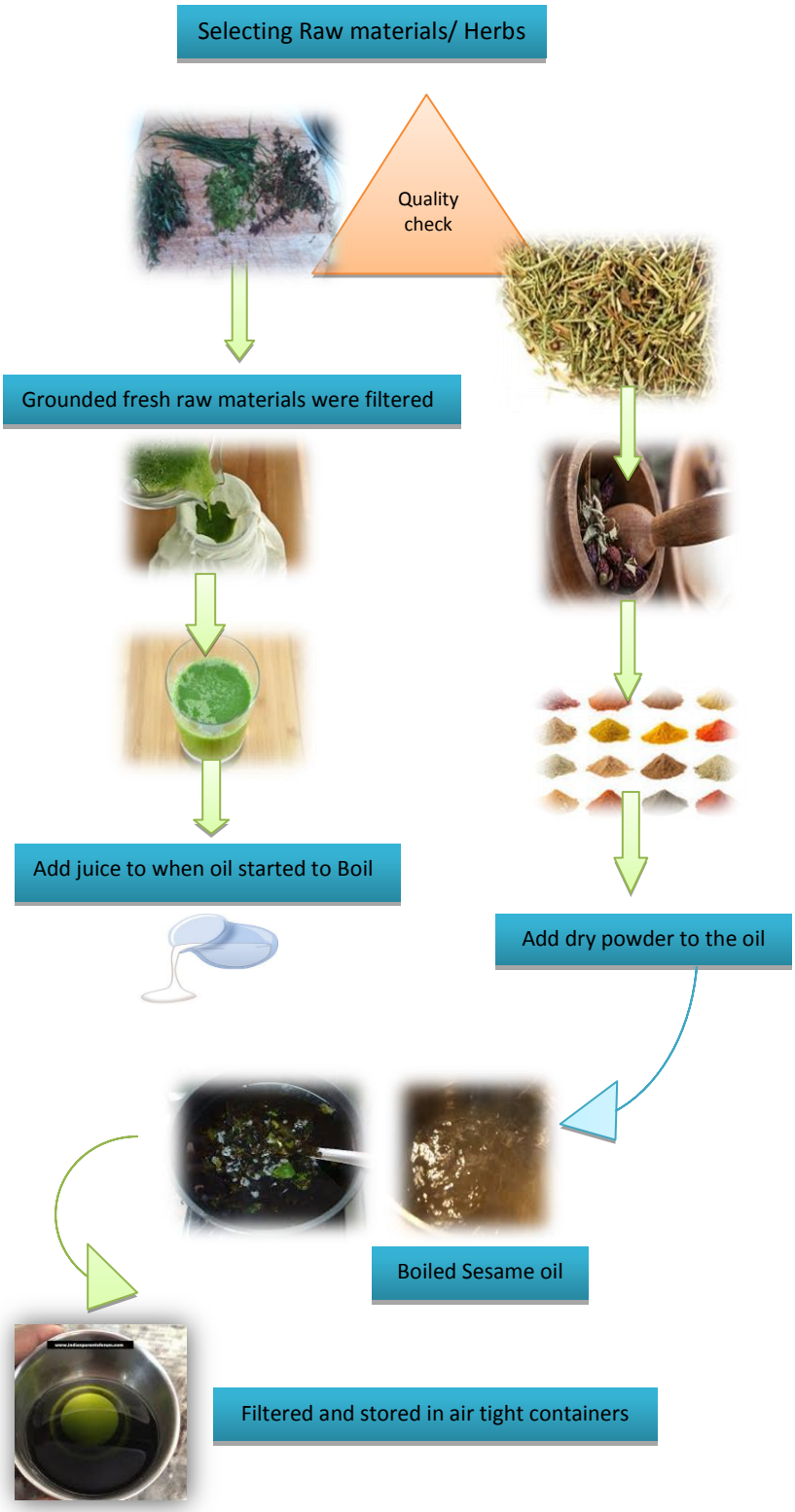


Fig. 1. Preparation of Kayathirumeni Thailam

2.5. Determination of saponification value

Weigh 1 gm. of oil and transfer into the round bottomed flask. Add 20 ml of 0.5 N alcoholic KOH solutions to the round bottomed flask. Follow the above procedure without taking oil for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point. (Page: 109) [7]

2.6. Determination of Peroxide value

Transfer approx. 3.0 g of the sample, accurately weighed, into a 250 ml Erlenmeyer flask with glass stopper. Add 50 ml of the glacial acetic acid: chloroform 3:2 solvent mixtures and saturated potassium iodide solution, 1 ml, freshly prepared and allow reacting for 60 seconds ± 1 second and shaking thoroughly during this period. Then add water, 100 ml and shake. Titrate with 0.01 mol/l sodium thiosulfate solution, using 1 ml starch solution indicator. The indicator should be added towards the end of the titration but while the pale straw colour is still present. During titration shake until the blue colour disappears. Carry out a blank titration under the same conditions. (Page: 109) [7].

2.7. Determination of Iodine value

Weigh 0.5 gm. of oil and transfer into Iodine flask. Add 10 ml of chloroform and warm slightly and cool for 10 minutes. Add 25 ml of Wiji's solution in the same flask and shake well. Then allow the flask to stand for half an hour in refrigerator. Add 10 ml of KI solution and after that titrate the solution against 0.1 N Sodium thiosulphate solutions until the appearance of yellow colour. Add 1 ml of starch indicator and again titrate against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample (i.e. Oil) and note the corresponding reading for blank titration (Page: 102) [7].

2.8. Rancidity Test

Measured 5 ml of testing oils in clean air dried test tube. Add 5ml of conc. HCL in the testing oils. Followed by add 5ml of 1 % phloroglucinol solution. Shake the test tubes until the all the solutions get mixed well. Allow the set-up for 10 minutes. If the oil is rancid, a Pinkish red lower layer will developed. The absence of pinkish red colour in lower acid layer is the indication of absence of rancidity (page: 89).[8]

2.9. Development of Thin Layer Chromatography (TLC) fingerprints of Medicated oil

The extraction was done by adding 100 ml of 90% v/v aqueous methanol to 50 ml of medicated oil in a conical flask and stirred for 1 h using a magnetic stirrer. After stirring it was covered with aluminium foil and kept in deep freezer at −20° for 2 d. At this stage the oils were seen to be almost solidified, the top methanol layer was filtered immediately through what man No. 40 filter paper [9]. This methanol extract was used for further TLC finger print analysis.

2.10. Test for Microbial load

About one gram sample was mixed with sufficient sterile water (the dilution blank) to make one hundred milliliters of stock solution. Serial dilutions of the stock solution were made with the same dilution blank. One milliliter aliquot of sample from each dilution was added to sterile petri plates in triplicate, followed by molten cooled nutrient agar medium. Plates were incubated for a period of 24 - 48 hours at a temperature of 28±2°C. The colonies were counted on a Quebec colony counter [10].

2.11. Test for heavy metals

Sample preparation was carried out as per AOAC Method[11] Acid Digestion of Sediments, Sludge's and Soils. About 1g of sample was digested in a digestion vessel with 10 mL HNO_3 by heating to 95°C for about 3 minutes. The digested sample was made up to 100mL using Millipore water and used for testing. Lead and cadmium were quantitatively estimated using Atomic Absorption Spectrometry. Arsenic was estimated by Hydride Generation Technique using nitrogen as carrier gas. Mercury was determined by Cold Vapour Technique after reduction with stannous chloride using argon carrier gas[11].

3. RESULTS AND DISCUSSION

Generally, medicated oils have characteristic colour and odour due to existence herbs and other raw materials. Kayathirumeni thailam had the characteristic green colour was the mixture of twenty-five herbal juices and raw drugs. The characteristic odour present in the oil was due to react of herbs with coconut oil. The present study indicates the characteristic colour, odour, appearance, touch and clarity of the oil was similar to the classical preparation. (Table 1)

Table.1 Organoleptic evaluation of Kayathirumeni thailam

S.No	*Parameters*	*Results*
1	Colour	Light green
2	Odour	Aromatic
3	Appearance	Clear oil
4	Touch	Greasy substance

Refractive index (RI) is an important indicator of medicated oil. It is widely used to find a particular substance and confirm its purity or measure. Commonly RI varies with temperature and wavelength. Changes in the basic constituents may occur due to break down/ activation of certain compounds. The present study found the average value ranges between 1.458 and 1.501 will be served as standard RI value of Kayathirumeni thailam. Since the values are similar to the other classical oil, it seems there is no room for other adulterants substance present in the oil[12]. (Table.2)

Acid value is also called as neutralizing value. The amount of KOH in milligram required to neutralize the acid present with any compound. The acidity increases with oxidation because TGA is converted to fatty acid and glycerol. There is a direct relationship between the acid value and rancidity. The present study revealed the acid value of the entire sample below 3 % indicated the better quality[13&14]. (Table.2)

Table.2. Physico chemical Characters of Kayathirumeni thailam

S. No	*Parameters*	*Results*
1	Acid Value (mg KOH g^{-1})	NMT 3.5
2	Iodine Value (mg KOH g^{-1})	145.35 ± 2.5
3	Saponification Value (mg KOH g^{-1})	253.54 ± 3.0
4	Peroxide value (mg KOH g^{-1})	1.25 ± 0.5
5	Kries test	Absence of pink colour
6	Specific Gravity	0.874 to 0.899
7	Refractive Index	1.458 to 1.499
8	oil soluble extractive value	NTL 4 %
9	Thin layer chromatography	5 to 11 spots

Saponification number is some of milligram of KOH required to saponify one gram of fats in specified conditions. The length of the fatty acid chain is inversely proportional to the saponification number[15.] The saponification range of Kayathirumeni falls between 253.54 ± 3.0. The observation shows medium chain fatty acid as main components. Medium chain triglycerides are considered as good biologically inert source which are easily metabolized by the human body. (Table.2)

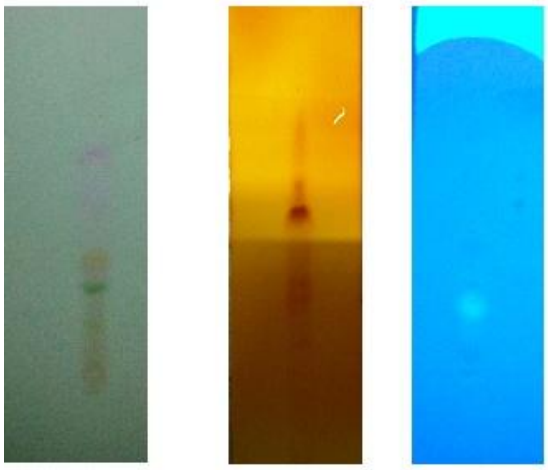
Peroxide values are mille equivalents of peroxide per kg of fats. Peroxide (O_2) is intermediate products of fats oxidation and breakdown rapidly to aldehyde, ketone and other products. The peroxide value of present study shows the lower range than the base oil. (Table.2) to setup the rate of oil oxidation is very important fact to quality achievement and product status to finalize the shelf life[16]. The present study reveals that the lower peroxide values of the oil shows the greater shelf life.

Iodine value is to measure the degree of unsaturation in oil and it could be used to quantify the amount of double bond present in the oil which reflects suspension of oil to oxidation[17]. The standard iodine value of Kayathirumeni oil is 145.32 ±2.5. (Table.2)

Thin layer chromatography is one among the important tool to find out exist active constituents of plants which used to prepare medicated oil. The chromatography study of Kayathirumeni thailam had done with Battery of solvent system. Among them Toluene: Ethyl acetate (7:3) and n-Hexane: Ethyl acetate (7.2:2.9) had better resolution. The present findings revealed and conform the active constituents' presents in the medicated oil. Planer chromatography results and Rf values presented in Table.3 & Fig.3.

Table. 3. Rf values of Kayathirumeni in different mobile phase

S. No	*Mobile Phase*	*Detecting Agent*	*Rf values*
1.	n-Hexane : Ethyl acetate(7.4:2.6)	Iodine Chamber	0.06,0.09,0.24,0.30, 0.36,0.44,0.49,0.58, 0.67,0.78,0.87
2.	n-Hexane : Ethyl acetate (7.4:2.6)	UV Chamber	0.30,0.44,0.58
3.	n-Hexane : Ethyl acetate (7.4:2.6)	10 % Sulphuric acid	0.09,0.44,0.58,0.61,0.87
4.	Toluene : Ethyl acetate (7:3)	UV Chamber	0.09,0.16,0.23,0.30,0.44,0.58, 0.61,0.67,0.80



10 % sulphuric acid iodine chamber UV-chamber

Fig 3: Thin layer chromatography of Kayathirumeni thailam

Microorganisms are known to cause chemical changes in oil that lead to deterioration the quality of chemical compounds[18]. Frying and cooking of oil will cut the microbial load to the least level. Nevertheless oil prone to contaminate by microorganism found in the environment, raw materials and equipment used for processing.

To avoid such instance microbial load should be checked in the formulations. The present investigation found microbial load falls within the least acceptable microbial level required by WHO which stipulate the most allowable amount of organism. (Table.4)

Table.4 Microbiology analysis of Kayathirumeni

S.No	*Parameters*	*Results*
1.	Total Aerobic bacterial Count	NMT 10^3 CFU/ml or g
2.	Yeast and Mould Count	NMT 10^2 CFU/ml or g
3.	*E.coli*	Nil
4.	*Salmonella spp.*	Nil
5.	*Pseudomonas aeruginosa*	Nil
6.	*Staphylococcus aureus*	Nil

There was myth about Siddha drug preparation has heavy metals which is harmful to human system. In order to proof the misconception, Heavy metal analysis had done. The finding reveals that major metals such as Cd, L, Ar, & M were not present in the detectable limits. It shows the Thailams are safe and free from metal toxicity (Table.5).

Table.5 Heavy metals analysis of Kayathirumeni

S.no	*Parameters*	*Standards (AYUSH)*	*Results (PPM)*
1.	Lead	NMT 10 mg/kg	0.51 mg/kg
2.	Mercury	NMT 1 mg/kg	BLQ (LOQ-0.25)
3.	Arsenic	NMT 3 mg/kg	BLQ (LOQ-0.17)
4.	Cadmium	NMT 0.3 mg/kg	BLQ (LOQ-0.0125)

4. STATISTICAL ANALYSIS

All experiments were carried out at least in three separate experiments (Triplicates). The results were expressed as means ± SD and the mean value were plotted in all figures and Pearson correlation coefficient (r^2) were calculated using data of each triplicate. All the analysis was carried out using SPS 12 software.

5. CONCLUSIONS

The need of quality control method for Siddha drug is an essential one due to commercialization of Siddha pharmacies and the traditional drugs include under Drug and Cosmetic act (DCA). Data obtained from the above limits are average value of different batches of thailam. The analytical data which was obtained by the present study will be served as a standard parameter for Kayathirumeni thailam because the standardization attempted for the first time.

REFERENCES

1. Organisation Mondiale De La Sante, Quality control methods for medicinal plant materials. World Health Organisation; **1992,** 159.
2. Agarwal S & Singh RH. Proceedings of International Congress, Ayurveda, 28–30th Jan; **2002.**
3. Thiagarajan R, Gunapadam part 2 & 3 Dept of Indian Medicine and Homoeopathy **1998,** 156.
4. Rajaram TK. Formulary of varma medicine. A.T.S.V.S Siddha medical college & hospital, Kanyakumari; **2008**, 257
5. Kumar KJ. Bottle Necks in Standardization of Traditional System of Medicines, *Research J. of Med. Plant* **2011**, (10).3923.
6. Anonymous, The Siddha Pharmacopoeia of India I vol I, Government of India, Ministry of Health and Family welfare, Department of ISM & H, **2001**.
7. Anonymous, India Pharmacopeia I Vol I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, **2014.**
8. Anonymous, *Manual of Methods of Analysis of Foods (Oils and Fats).* Food safety and standards authority of India, Ministry of health and family welfare, **2012**; P.89.
9. Lahorkar P, Ramitha K, Bansal V & Anantha Narayana DB. A Comparative Evaluation of Medicated Oils Prepared Using Ayurvedic and Modified Processes. *Indian .J. Pharmaceutical Sci*, 71, **2009,** 656-662. CrossRef , PMid: 20376219 PMCid: PMC2846471.

10. Anonymous, Manual of analysis of foods: Mycotoxins, Food Safety and Standards Authority of India, New Delhi, **2012.**
11. Garthersburg MD, Official Methods of Analysis of AOAC International, AOAC International, (Official Methods USA), **2000**, (986):15.13.
12. Kinsler, Lawrence E. Fundamentals of Acoustics. John Wiley; **2000,** p. 136.
13. Anonymous, India Pharmacopeia I Vol I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, **1999,** A-78.
14. Khandelwal KR, Practical Pharmacognosy Techniques and experiments, Nirali Publication, Pune, **2009,** (9):157-161
15. Kumara KV & Nishteswar K, Analytical study of Vrunashodana Taila: A wound healing medicated oil. *Int. J. Res. Ayurvecid Pharm*, **2011**, 1481-1492.
16. Kumaradharmasena LSP, Arawwawala LDAM, Fernando PIPK, Peiris KPP, Kamal SV. Quality assessment of Mustadi Taila: An Ayurvedic oil as a remedy for Dental Caries (Krimi Danta). *Journal of Pharmacognosy and Phytochemistry*, **2015**, 4(3) 21-24.
17. Danlami, U & David, BM, Physico-Chemical Properties and antioxidant potentials of *Daniella oliveri* seed oil. *Res.J.Eng. Applied Sci*, 1, **2012**: 389-392.
18. Larry SB, *Food and Beverages mycology* II. New York, Van Nostrand Reinhold. **1987**, 259.

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