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Research Article

A COMPARATIVE PHYTOCHEMICAL ANALYSIS OF GENUINE AND MARKET SAMPLE OF "*GAJAPIPPALI*" W.S.R TO *SCINDAPSUS OFFICINALIS* SCHOTT (ARACEAE)

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ABSTRACT

Gajapippali (*Scindapsus officinalis* Schott.) is one of the plants used in Indian system of medicine belongs to family Aracea. In the present study physicochemical analysis and preliminary phytochemical screening of fruits of genuine and market sample have been carried out. The total ash is found to be 9.67%, 6.74%, 6.6%, the acid insoluble ash is found to be 1.12%, 0.57%, 0.7%, the water soluble ash is found to be 16.75%, 19.59%, 19.6% respectively both in genuine and market sample. The alcohol soluble extractive value is found to be 11.53%, 9.56%, 9.6%, the aqueous soluble extractive value is found to be 19.65%, 18.91%, 18.94%, the petroleum-ether extractive value is found to be 4.59%, 3.65%, 3.7% respectively both in genuine and market sample. The moisture content is found to be 6.4%, 3.6%, 3.5% respectively both in genuine and market sample. The p^H value is found to be 8.1, 6.8, 6.8 respectively both in genuine and market sample. Preliminary phytochemical screening of successive extracts of fruit reveals the presence of various secondary metabolites such as Alkaloids, glycosides, saponins, tannins, steroids, phenolic compounds, amino acids, proteins and carbohydrates. The TLC and HPTLC finger print study also carried out in both genuine and market sample. By the study I have sure that this work will be helpful to further standardization of the drug.

KEYWORDS: *Gajapippali*, *Scindapsus officinalis* Schott. Phytochemical analysis, TLC, HPTLC finger print.

INTRODUCTION

Gajapippali (*Scindapsus officinalis* Schott) is a large stout climber with woody stems when old, nodes often with fleshy areal roots frequently climbing on trees and rocks in damper valleys. Spathe oblong, 10-15 cm long, terminating in a long acumen, green outside, yellow within, deciduous. Spadix stout, nearly as long as the spathe, elongating to 15-22.5 cm in fruit with densely packed prismatic truncate ovaries grooved below, the short 4-celled anthers in the grooves. Stigma elongate, hemispheric in fruit. The fruit of *Scindapsus officinalis* is considered as *Gajpippali* in Ayurveda. Ancient and modern literature have mentioned that the fruits of *Gajapippali* possess Anthelmintic, Antibacterial, Anti-inflammatory and Analgesic, Anti-asthmatic, Antimicrobial, Cytostatic, Anti-oxidant, Aphrodisiac, Galactagogue activities.[1]

To the best of our knowledge, the fruits of the plant have not been analysed phytochemically by any Ayurvedic scholar for physicochemical parameters and preliminary phytochemical evaluation. These facts justify interest of scholar in this study.

MATERIAL AND METHODS

Plant Materials

The plant material taken for study is:

- ***Gajapippali* Fruits (Genuine and Market samples)**

Collection of genuine samples

The genuine samples were collected by scholar himself in presence of guide and co-guide from their natural habitat after identifying the source of plant as per standard description from the Sal forest of Lachiwala, near to Dehradun, Uttarakhand, and from the Satakosia forest of Dist- Mayurbhanja, Odisha, India.

For study purpose the genuine sample collected from Satakoshia forest, Odisha dated on 26/12/2015 was coded as $S_{(1a)}$ and from Lachiwala, Dehradun, Uttarakhand on dated 17/11/2015 coded as $S_{(1b)}$.

Collection of Market samples

The market samples are collected by scholar himself from the different major markets of India.

The collected market samples were coded as given below.

1- Market sample of Khari-Babli bazaar, New Delhi. Coded as $S_{(2)}$

2- Market sample of Haridwar, Uttarakhand. Coded as $S_{(3)}$

3- Market sample of Jaipur, Rajasthan. Coded as $S_{(4)}$

4- Market sample of Bhubaneswar, Odisha. Coded as $S_{(5)}$

Following points were kept in mind during collection of market samples

1. Markets samples were collected as such and not verified/discussed on spot.

2. Sample purchased were properly collected, labelled, stored and subjected to evaluation.

Genuine Sample

Sample code	Place of collection	Date of collection	Collector
$S_{(1a)}$	Satakoshia forest, Dist-Mayurbhanj, Odisha	26/12/2015	Scholar
$S_{(1b)}$	Lachiwala, Dehradun, Uttarakhand	17/11/2015	Guide, Co- Guide & Scholar

Market Sample

Sample Code	Market	Date of Purchasing	Collector	Price/ kg
$S_{(2)}$	New delhi	07-09-2015	Scholar	Rs 250/kg
$S_{(3)}$	Haridwar	04-10-2015	Scholar	Rs 300/kg
$S_{(4)}$	Jaipur	10-12-2015	Scholar	Rs 280/kg
$S_{(5)}$	Bhubaneswar	20-10-2015	Scholar	Rs 240/kg

Test

- Moisture content
- pH
- Alcohol Extractive Value
- Aqueous Extractive Value
- Petroleum Ether Extractive Value
- Total Ash
- Acid Insoluble Ash
- Water Soluble Ash
- Qualitative analysis of Phytochemicals
- TLC
- HPTLC finger prints
- Anti Oxidant Activity

Name of the Institution- The complete research has been perform in the following institution.

Institute of Biomedical and Industrial Research, Reg. No. - 813 Jaipur/2011-12, CPCSEA Registration No. - 1737/PO/Rc/S/14/CPCSEA, 5/35- Vidhyadhar nagar, Jaipur, Rajasthan-302023.

Methods[2, 3]

Determination of moisture content

Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105º for 5 hours, and calculate weight of sample for every 30 minute, until the weight of the sample were constant, no variation of weight are recorded. This sample was allowed to cool at room temperature in desiccators for 1 hour before weighing.

Determination of pH value

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per litre.

- The pH of a given solution is measured by using digital pH meter.
- The instrument is switched on. Leaved for some time unless or on the board requirement of different pH solution appears.
- Buffer solution is taken in the beaker and the electrode is dipped in it. Same procedure is repeated for the other buffer solutions after washing the electrode thoroughly with distilled water.

The sample is taken (10% aqueous solution) and dips the electrode in it and note the value of pH.

Determination of Alcohol Soluble Extractive

It was taken Macerate 5 gm of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 1050, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Proceed as directed for the determination of alcohol-soluble extractive, using distilled water instead of ethanol.

Determination of Petroleum Ether Soluble Extractive (Fixed Oil Content)

Transferred a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with solvent ether (or petroleum ether, b.p. 40º to 60º) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filtered the extract quantitatively into a tarred evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

Determination of Total Ash

The total ash method is design to measure the total amount of material remaining after ignition. This include both physiological ash which derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface. Silica Crucible was cleaned, dried well, labelled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

Determination of acid insoluble Ash

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash

(Prepared by method 2), with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccators and weighed. Calculated the percentage of acid - insoluble ash with reference to the air - dried drug.

Water soluble Ash

Water - soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash (Prepared by method 2) for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 4500 C. It was subtracting the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculated the percentage of water – soluble ash with reference to the air - dried drug.

Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid.

Chromatography plates

For chromatography T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Merck's) were used. (Each plate dimension is 10 cm long and 2 cm width).

Activation of pre-coated Silica gel G60F254

Dry in hot oven at 105^0 C for one to two hour

Test sample

Alcoholic extract

Preparation of mobile solution

Chloroform**:** Methanol (1:1)

Sample application

Sample was applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

Visualization

- 5% Methanolic-Sulphuric acid reagent and heating the plate for ten minutes at 110°C.

Rf. Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Antioxidant Activity

Evaluation of the Antioxidant Activity Determination of the free radical scavenging activity of the different extracts was carried out using a modified quantitative DPPH (1, 1-diphenyl-2-picrylhydrazyl; Sigma Aldrich, St. Louis, MO, USA) assay. Various concentrations of sample extracts in methanol were prepared (1000, 500, 250, and 100 µg·ml–1). Gallic acid was used as a positive control at concentrations of 100, 50, 25, and 10 µg·ml–1. Blank samples were run using 1 ml methanol in place of the test extract. One ml of 0.2 mm DPPH in methanol was added to 1 ml of the test solution, or standard, plus 1 ml of methanol for dilution and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 517 nm. Inhibition of free radical in percent (I%) was calculated according to the following

I%= {(A0-A1)/A0} X 100

TLC plate development-pre-saturated CAMAG twin through chamber.

HPTLC Finger prints

High performance thin layer chromatography (**HPTLC**) is an enhanced form of thin layer chromatography (TLC). Widely used instrument for HPTLC is from CAMAG, Switzerland. It provides automated sample application (loading), plate development, detection and documentation.

HPTLC finger print profile of Genuine sample $S_{(1b)}$ & Market sample $S_{(2)}$

Extract use- Methanol extract

Application – Linomate 5 application (CAMAG)

Volume applied- 6.0µl

Solvent system- Toluene: Ethyl acetate: Formic acid: Ethanol (6:4.0.3:0.4), Scan

Wave length- 254nm, 366 nm

Results

The present work encloses detailed studies on physicochemical analysis in dried fruit's ash, preliminary phytochemical screening, HPTLC finger print analysis and antioxidant activity on successive extracts of both genuine and market samples.

Physio-Chemical Analysis

Quantitative Test of Genuine and Market Sample

Moisture content

S.No	Sample	Moisture Content	Standard as per API
1.	$S_{(1a)}$ (Genuine)	6.4%	Not mention
2.	$S_{(3)}$ (Haridwar market)	3.6%	
3.	$S_{(4)}$ (Jaipur market)	3.5%	

Determination of aqueous soluble extractive value

S.No	Sample	Aqueous Extractive Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	19.65%	Not less than 11%
2.	$S_{(3)}$ (Haridwar market)	18.91%	
3.	$S_{(4)}$ (Jaipur market)	18.94%	

Determination of alcohol soluble extractive value

S.No	Sample	Alcohol Extractive Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	11.53%	Not less than 3%
2.	$S_{(3)}$ (Haridwar market)	9.56%	
3.	$S_{(4)}$ (Jaipur market)	9.6%	

Estimation of petroleum-ether extractive value

S.No	Sample	Petroleum-Ether Extractive Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	4.59%	Not Mention
2.	$S_{(3)}$ (Haridwar market)	3.65%	
3.	$S_{(4)}$ (Jaipur market)	3.7%	

Estimation of acid-insoluble ash

S.No	Sample	Acid-insoluble Ash Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	1.12%	Not more than 1.5%
2.	$S_{(3)}$ (Haridwar market)	0.57%	
3.	$S_{(4)}$ (Jaipur market)	0.7%	

Estimation of water soluble ash value

S.No	Sample	Water Soluble Ash Value
1.	$S_{(1a)}$ (Genuine)	16.75%
2.	$S_{(3)}$ (Haridwar market)	19.53%
3.	$S_{(4)}$ (Jaipur market)	19.6%

Estimation of total ash value

S.No	Sample	Total Ash Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	9.76%	Not more than 14%
2.	$S_{(3)}$ (Haridwar market)	6.74%	
3.	$S_{(4)}$ (Jaipur market)	6.6%	

P^H value

S.No	Sample	pH Value	Standard as per API
1.	$S_{(1a)}$ (Genuine)	8.1	Not mention
2.	$S_{(3)}$ (Haridwar market)	6.8	
3.	$S_{(4)}$ (Jaipur market)	6.8	

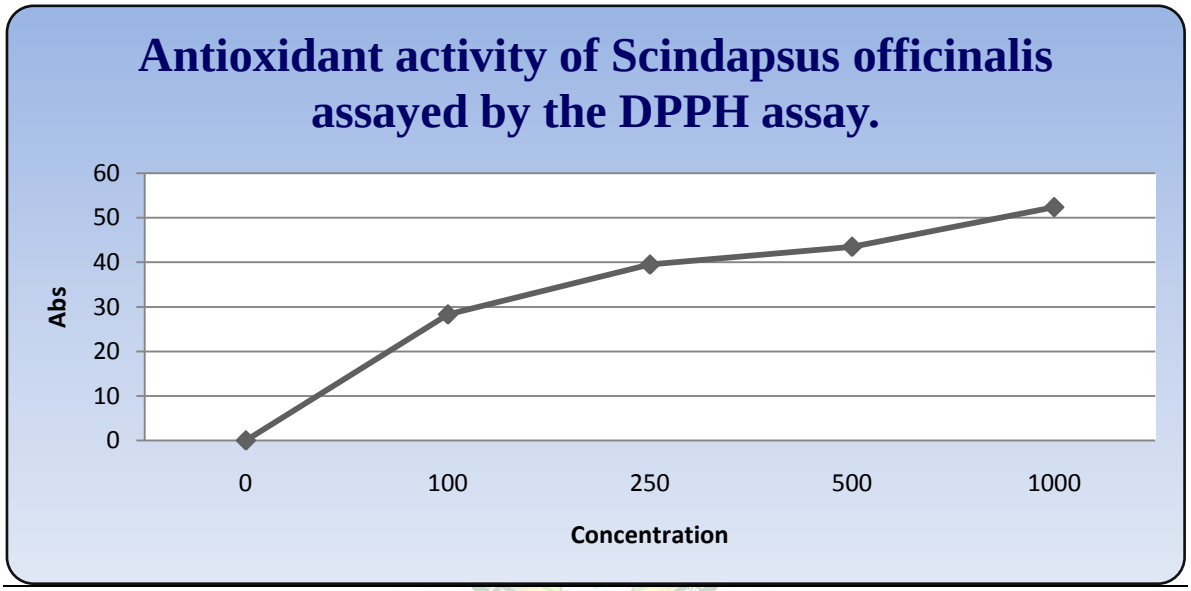
Thin layer chromatography (TLC) of genuine and market sample of Gajapippali fruit.

TLC	$S_{(1a)}$ (Genuine)	$S_{(3)}$ (Haridwar)	$S_{(4)}$ (Jaipur)
R_f Value	0.65, 0.74, 0.92	------	-------

Anti-Oxidant Activity of *Gajapippali* fruit.

Antioxidant activity of *Scindapsus officinalis* Schott ($S_{(1a)}$) assayed by the DPPH assay:

Conc. of extract µg/ml	Methanol Extract of *Scindapsus officinalis*	Conc. of standard µg/ml	Gallic acid
1000	52.4 %	100	90.5 %
500	43.5 %	50	83.2 %
250	39.5 %	25	76.4 %
100	28.3 %	10	65.1 %



HPTLC Finger printing

HPTLC Finger printing of genuine sample ($S_{(1b)}$) and market sample ($S_{(2)}$) was performed at NBRI, Lucknow. The details are as follows

HPTLC finger print profile of Genuine sample ($S_{(1b)}$)

Extract use- Methanol extract
Application– Linomate 5 application (CAMAG)
Volume applied- 6.0µl
Solvent system- Toluene: Ethyl acetate: Formic acid: Ethanol (6:4.0.3:0.4), Scan
Wave length- 254nm, 366 nm
TLC plate Development-Pre-saturated CAMAG twin through chamber.

Summarized table of HPTLC Finger print test: ($S_{(1b)}$)

Peak	Start Position	Start Height	Max. Position	Max. Height	Max. %	End Position	End Height	Area	Area %	Assigned Substance
1.	0.00 R_f	0.6 AU	0.04 R_f	362.2AU	52.17%	0.08 R_f	11.6AU	5573.7AU	26.88%	Unknown
2.	0.10 R_f	8.1 AU	0.12 R_f	27.7AU	4.00%	0.15 R_f	12.6AU	777.8AU	3.75%	Unknown
3.	0.17 R_f	14.2AU	0.18 R_f	15.3AU	2.20%	0.20 R_f	2.1AU	250.0AU	1.21%	Unknown
4.	0.31 R_f	7.3 AU	0.32 R_f	14.6AU	2.10%	0.33 R_f	0.8AU	225.2AU	1.09%	Unknown
5.	0.42 R_f	2.6 AU	0.48 R_f	65.3 AU	9.41%	0.51 R_f	12.7AU	2376.1AU	11.46%	Unknown
6.	0.51 R_f	13.0AU	0.53 R_f	24.2AU	3.49%	0.54 R_f	19.4AU	532.7AU	2.57%	Unknown
7.	0.57 R_f	25.3AU	0.58 R_f	39.2AU	5.64%	0.63 R_f	4.6AU	1275.5AU	6.15%	Unknown
8.	0.66 R_f	3.9 AU	0.67 R_f	13.9 AU	2.00%	0.69 R_f	1.6 AU	174.6AU	0.84%	Unknown
9.	0.80 R_f	32.2AU	0.89 R_f	101.5AU	14.61%	0.93 R_f	35.8AU	8921.0AU	43.03%	Unknown
10.	0.95 R_f	27.8AU	0.96 R_f	30.4AU	4.38%	0.98 R_f	10.6AU	625.3AU	3.02%	Unknown

HPTLC finger print profile of Market sample ($S_{(2)}$)

Extract use - Methanol extract

Application - Linomate 5 application (CAMAG)

Volume applied- 6.0µl

Solvent system- Toluene: Ethyle acetate: Formic acid: Ethanol (6:4.0.3:0.4), Scan

Wave length- 254nm, 366 nm

TLC plate Development-Pre-saturated CAMAG twin through chamber.

Summarized table of HPTLC Finger print test: ($S_{(2)}$)

Peak	Start Position	Start Height	Max. Position	Max. Height	Max. %	End Position	End Height	Area	Area %	Assigned Substance
1.	0.00 R_f	1.4AU	0.04 R_f	741.1AU	66.11%	0.09 R_f	26.0AU	11120.4 AU	42.55%	Unknown
2.	0.10 R_f	25.1 AU	0.13 R_f	85.2AU	7.60%	0.18 R_f	10.1AU	3429.3 AU	13.12%	Unknown
3.	0.35 R_f	3.4AU	0.37 R_f	22.4AU	2.00%	0.38 R_f	5.0AU	265.1AU	1.01%	Unknown
4.	0.39 R_f	7.4 AU	0.43 R_f	88.6AU	7.90%	0.47 R_f	9.9AU	2923.9 AU	11.19%	Unknown
5.	0.47 R_f	10.1 AU	0.48 R_f	24.0 AU	2.14%	0.48 R_f	19.7AU	249.2AU	0.95%	Unknown
6.	0.51 R_f	13.8AU	0.52 R_f	42.6AU	3.80%	0.55 R_f	4.8AU	1018.1 AU	3.90%	Unknown
7.	0.64 R_f	2.5AU	0.65 R_f	13.4AU	1.19%	0.66 R_f	4.5AU	142.5AU	0.55%	Unknown
8.	0.74 R_f	0.3 AU	0.76 R_f	19.5 AU	1.74%	0.80 R_f	1.5 AU	488.7AU	1.87%	Unknown
9.	0.81 R_f	3.3AU	0.89 R_f	84.4AU	7.53%	0.95 R_f	9.7AU	6499.5AU	24.87%	Unknown

DISCUSSION

Physicochemical Study: Genuine $S_{(1a)}$ Market sample $S_{(3)}$, $S_{(4)}$

The physicochemical study was done on the drug following the validated methods as per API.

- Moisture content in genuine sample was 6.4% and in market sample of Haridwar and Jaipur was 3.6% and 3.5%. An excess of water in drug encourage microbial growth, a presence of fungi or insects and deterioration following hydrolysis.
- The ash value is the indicator of the presence of inorganic & earthy matter in the plant. It is used to determine the purity of a crude drug and to establish the identity of it. The higher ash value is suggestive of thermo-non labile/ heat stable or inorganic constituents.
- Total ash content found in genuine sample was 9.76% and in market sample of Haridwar and Jaipur was 6.74% & 6.6% which was within range (Standard as per API: Not more than 14%).
- Acid insoluble ash shows presence of siliceous material and heavy metals. Acid–insoluble ash in genuine sample was 1.12% and in market sample of Haridwar and Jaipur was 0.57% & 0.7 %.(Standard as per API: Not more than 1.5%).
- Water Soluble Ash shows Quantity of Inorganic Substance. Water soluble ash found in genuine sample was 16.75% and in market sample of Haridwar and Jaipur was 19.53% & 19.6%.
- Extractive value shows soluble content present in samples.
- Water soluble content present in genuine sample was 19.65% and in market sample of Haridwar and Jaipur was 18.91% & 18.94% (Standard as per API not less than 11%).
- Alcohol soluble Content present in genuine sample was 11.53% and in market sample 9.56% & 9.6% (Standard as per API not less than 3%). Petroleum-ether extractive value present in genuine samples was 4.59% and in market sample 3.65% & 3.7%.
- pH is a method of quantity analysis of acidic and basic nature of drug. pH of genuine sample was 8.1and that of market sample 6.8 & 6.8.

Thin layer chromatographic study

Thin layer Chromatography is a tool for separation and identification of chemical constituent.

TLC of genuine and market sample was done with mobile solution Chloroform: Methanol in the ratio of 1:1. In sample $S_{(1a)}$ R_f Value 0.65, 0.74, 0.92 was found and in sample $S_{(3)}$ & $S_{(4)}$ no any spot found in mobile.

According to Chromatography analysis no any chemical similarity found in between sample $S_{(1a)}$ and $S_{(3)}$, $S_{(4)}$ i.e. genuine sample and market sample.

HPTLC Finger print analysis

HPTLC finger print analysis of the genuine and market sample of *Gajapippali* fruit extracts showed the presence of possible number of components. This study demonstrates the consistent quality of chemical constituents.

- The results from HPTLC finger print of genuine sample ($S_{(1b)}$) scanned at wavelength 254nm and 366nm for methanol extract shows ten polyvalent phyto-constituents and corresponding ascending order of R_f values starting from 0.08 to 0.98 in which highest concentration of the phyto-constituents was found to be 52.17% and its corresponding R_f value was found to be 0.08.
- The results from HPTLC finger print of market sample of New Delhi ($S_{(2)}$) scanned at wavelength 254nm and 366nm for methanol extract shows nine polyvalent phyto-constituents and corresponding ascending order of R_f values starting from 0.09 to 0.95 in which

highest concentration of the phyto-constituents was found to be 66.11% at corresponding R_f value 0.09.

CONCLUSION

The plant drug *Gajapippali* (*Scindapsus officinalis* Schott.) belongs to family Araceae. In the present study Physicochemical analysis, Phytochemical screening, Antioxidant and HPTLC finger print of the fruit have been carried out. The outcomes of physicochemical study on the fruit play a significant role in setting the standards for fruit. The plant has both inorganic and organic ingredient. The inorganic fraction of the medicinal plant contains mainly mineral elements viz; calcium, chloride, chromium, copper, iron, lead magnesium, manganese, phosphorous, potassium, selenium, sodium and zinc. These mineral essentials may be associated with the various vital processes of the body. Preliminary phytochemical screening of successive extracts of fruit reveals the presence of secondary metabolites which may be responsible for a variety of pharmacological activities of fruit. HPTLC finger print analysis of *Gajapippali* fruit extracts showed the presence of possible number of components and demonstrates the consistent quality of chemical constituents. The fruit also shows a significant free radical scavenger activity. By the study we are confident that this work will be helpful for further standardization of the drug in future.

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Figure 1: Genuine Samples Collected from Different Natural Sources

Genuine sample of Odisha S(1a)

Genuine sample of Uttarakhand S (1b)

Figure 2: Market samples are collected from different major markets of India

Market Sample of Delhi S(2)

Market Sample of Haridwar S(3)

Market Sample of Jaipur S (4)

Market Sample of Odisha S(5)