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Comparative Physicochemical Evaluation of Fruits and Antidepressant Potential of Volatile Oils of Fruits of Local *Piper* Species

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ABSTRACT

In this study an attempt is made to evaluate physicochemical properties comparatively for the fruits of different *Piper* species available in the Mumbai region. The fruits of five species, viz. *Piper betle* Linn, *Piper cubeba* Linn. f., *Piper retrofractum* Vahl, *Piper longum* Linn and *Piper nigrum* Linn were evaluated comparatively for physicochemical properties, viz. Ash Value, Extractive Value, Loss on Drying, Mucilage Content, Crude Fibre Content, Volatile Oil Content and Piperine Content by Spectroscopic method. At the same time an attempt is made to evaluate antidepressant potential comparatively for the volatile oils of mentioned species, using forced swimming method, on albino mice with fluoxetine as standard antidepressant drug.

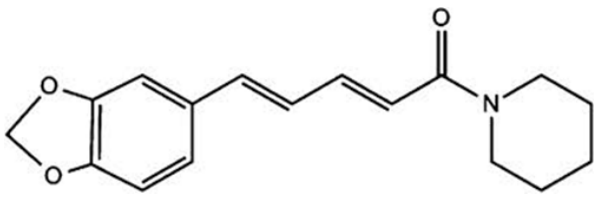
Key words: *P. betle* Linn, *P. cubeba* Linn. f., *Piper retrofractum* Vahl, *P. longum* Linn. *P. nigrum* Linn. Clevenger Apparatus, UV-Spectrophotometer.

INTRODUCTION

Piper Linn., belonging to family Piperaceae is a very large genus of shrub,rarely herbs and trees, distributed throughout the tropical and sub-tropical regions of the world. About 30 species of the genus in India and 700 species in the world have been reported, of which, *P. nigrum*, the Black Pepper and *P. betle* Linn., the Pan or Betel are widely cultivated[2]. Five species are used as herbal ingredients of Asian medicines and they are *P. betle* , *P. cubeba* Linn. f. (Cubebs), *P. retrofractum* Vahl syn. *P. chaba* Hunter non Blume (Java Long Pepper), *P. longum* Linn. (Indian Long Pepper) and *P. nigrum* Linn[3]. The leaf juice of *P. betle* is used as eye drop[4]. *P. cubeba* is used as antibacterial[5], expectorant[6] and as gastroprotective[7]. *P. longum* is used as bioavailability enhancer[8], digestive and in the treatment of bronchitis[9] and also as hepatoprotective agent[10]. Scientists have received US patent on obtaining a diabetes mellitus therapeutic agent from *P. longum*[11]. *P. nigrum* is

used as nervine tonic, and in the treatment of constipation, itching and flatulence[12]. Some of the Piper species contain a piperidine type alkaloid, piperine, which is a central nervous system depressant[13]. Most of the piper fruits contain volatile oils[14].

P.nigrum *P.longum* *P.chaba* *P.cubeba* *P.betle*



Piperine

IUPAC Name: 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine

MATERIALS AND METHODS

Collection of chemicals and plant material

For the present study fruits of all the five selected species of *Piper* (*P. betle* Linn, *P. cubeba* Linn. f., *Piper chaba (retrofractum)* Vahl, *P. longum* Linn & *P. nigrum* Linn), were collected from APMC Market, Vashi, Navi Mumbai, India. The fruits were identified, confirmed and authenticated by Prof. H.M. Pandit, Botany Dept., Khalsa College, Mumbai. Fruits of five *Piper* species were shade dried & ground to coarse powder. All reagents used in quantitative analysis and chemical investigation were of analytical grade and manufactured by E-Merck, Ranbaxy, Loba chemicals, S.D. fine chemicals and Yucca Enterprizes, Mumbai.

Physicochemical parameter

Determination of ash value[14]

Total Ash/ Water Soluble Ash/ Acid Insoluble Ash Value

For its detection, 2 gm of powdered material of each formulation and the individual ingredients of the powers were placed separately in a suitable tarred crucible of silica previously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash. The water soluble and acid insoluble ash value were then determined as per standard procedure.

Determination of Extractive Value[14]

Water/Alcohol/Ether Soluble Extractive Value

About 5g of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water/ alcohol/ ether respectively in three different closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. These were then filtered rapidly; taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water / alcohol / ether soluble extractive were calculated with reference to the air dried drug and is represented as% value. **Table 1**

Determination of Moisture Content[14]

About 2 gm of drug samples of each fruit was accurately weighed in a dried and tarred flat weighing bottle and dried at 105 ^{0}C for 5 hrs. Percentage was calculated with reference to initial weight. **Table 1**

Determination of Mucilage Content[15]

About 1 gm of drug samples of each fruit/ powder was accurately weighed and taken in a test tube. 10 ml of distilled water was added to it kept for 24 hours for maceration. On the next day fruit/powder solutions were filtered. The filtrate then treated with 5 ml of ethanol. The mucilage appeared in the ethanol which was then filtered through an already weighed watmann filter paper and difference in the weight was then calculated. **Table 1**

Determination of Crude Fibre Content[16]

About 2 gm of drug sample extracted with 50 ml of methylene chloride. Filtered and to the residue added 50 ml of dilute sulphuric acid, boiled for 30 minutes, filtered. The residue then washed with water. Ignited already weighed crucible and contents/residue in an electric muffle furnace at 600°C. Then difference in weight was calculated. **Table 1**

Determination of Volatile Oil Content[17]

About 100 gms of drug samples of each fruit was accurately weighed and transferred to a 500ml round bottom flask. Sufficient amount water was added and a Clevenger Apparatus then attached to the RBF with condenser and mixture was then heated for six hour for so as to isolate the volatile oil. The volatile oil of individual drug was then collected in graduated tube of Clevenger Apparatus and separated and stored in vials. **Table 1**

Determination of Piperine Content[18]

Instrument

UV Spectro-photometer: UV-1800-Shimatzu

Preparation of standard solution of Piperine

Accurately weighed Piperine (10 mg) was transferred in 100 ml volumetric flask and dissolved in & diluted to 100 ml with ethanol. The final solution contained 100 mg of the Piperine per ml of the solution.

Table 1: Physicochemical Evaluation of Fruits of Local *Piper* Species

S. No.	Name of the Drug	Ash Value			Extractive Value			Moisture Content	Fruit Mucilage Content	Crude Fibre Content	Volatile Oil Content	Piperine Content (gm percent)
		T.A.V.	W.S.A.V.	A.I.A.V.	W.S.E.V.	A.S.E.V.	E.S.E.V.					
1	*Piper nigrum*	10.8±0.4	8.8±0.2	1.0±0.1	2.5±0.1	6.2±0.2	5.8±0.2	1.80	3.8	0.35	1.6	1.70 gm %
2	*Piper longum*	5.7±0.2	3.2±0.1	1.5±0.1	18.5±0.4	6.8±0.2	9.8±0.2	1.35	7.9	0.45	0.2	0.30 gm %
3	*Piper chaba*	6.85±0.8	3.6±0.1	3.0±0.1	6.0±0.2	8.6±0.2	7.2±0.2	1.25	8.6	0.15	0.8	0.95 gm %
4	*Piper cubeba*	5.9±0.55	2.8±0.1	3.0±0.1	7.5±0.2	26.0±0.4	23.8±0.3	1.75	4.4	0.45	4.4	0.15 gm %
5	*Piper betle*	7.65±0.5	4.6±0.2	2.0±0.1	11.5±0..3	16.0±0.3	5.6±0.2	1.65	9.1	0.35	0.2	0.90 gm %

T.A.V.-Total Ash Value, W.S.A.V.-Water Soluble Ash Value, A.I.A.V.-Acid Insoluble Ash Value
W.S.E.V.Water Soluble Extractive Value, A.S.E.V. Alcohol Soluble Extractive Value & E.S.E.V.Ether Soluble Extractive Value

Preparation of Piperine extract of different Piper Extracts

Accurately weighed 1 gm of Piper fruit powder reflux with 40 ml of ethanol for 1 hour. Filtered the extract and re-reflux the marc I left with 30 ml of ethanol for another 1 hours. Filtered and combined the previous filtrate. Further reflux the mark II left with 20 ml of ethanol. Again filtered and combined filtrate with previous filtrates, finally make up the volume up to 100 ml with ethanol in a volumetric flask.

Preparation of calibration curve for Piperine

Standard solutions of Piperine (0.1, 0.2, 0.3, 0.4 and 0.5 ml) were pipetted in a series of five 10 ml volumetric flask so as to give concentration range 1-5 ug/ml The absorbance of the Piperine was measured at 342.6 nm against ethanol. The concentration and then the percentage of Piperine in different *Piper* fruits were calculated. **Table 1**

Antidepressant Activity[19-22]

Forced Swimming Method

The forced swimming test is adopted here is a modification of the method described by Porsolt et al. (1977). Mice were individually forced to swim for 15 min in glass cylinders (height: 20 cm, diameter: 14 cm), containing 10 cm of water at 25 °C, which is a pre-test, and then mice were removed and dried before being returned to cages. Then standard Fluoxetine and essential oil under tests were administered. Four hours later, mice were placed in the cylinders again for a 6-min test in the same system depicted above. The duration of immobility was recorded during the last 4 min of the 6-min testing period. Groups of 6 mice were treated with vehicle (10 mg/kg, p.o.), drug-treated groups (10 ml/kg, p.o.), Fluoxetine (1 mg/kg,i.p.). **Table 2**

RESULT AND DISCUSSION

In the exhaustive study of Piper fruits, the conclusions drawn are; (a) Piperine content in *Piper nigrum* is maximum followed by *Piper chaba* and minimum in *Piper cubeba.* Here an easy UV Spectroscopic method is used for Piperine determination (b) Volatile oil content is in maximum amount in *Piper cubeba* followed by *Piper nigrum* and *Piper chaba* and least in remaining two species of Piper. Here hydrodistillation method is used for isolation of Volatile oils. (c) Crude fibres are fat free organic substances which are insoluble in acid and alkaline media. The crude fibre content estimation showed that *Piper longum* and *Piper cubeba* have equal and maximum crude fibre content followed by *Piper nigrum* and *Piper betle* in equal amount. (d) The mucilage content in *Piper betle* was in maximum amount followed by *Piper chaba* and then Piper *longum*. It is revealed that circular or rounded shaped fruit do not contain mucilage in large amount. (e) It is found that moisture content in *Piper nigrum* is in maximum amount while in *Piper chaba* it in least amount.

All volatile oils of Piper fruits have shown more activity than the standard compound. Fluoxetine, which is a Selective Serotonin Reuptake inhibitor was taken as standard, therefore for all these oils which have shown comparable and more activity to that of Fluoxetine should be further studied with different models of Antidepressant Activity.

Table 2: Antidepressant Activity of Essential Oils of *Piper* Fruits: Forced Swimming Method

	Normal control	Standard	T-1	T-2	T-3	T-4	T-5
Body weight (g)	49.33 ± 2.06$^{\#}$	60.67 ± 3.53*	69.50 ± 1.78**	64.17 ± 2.17**	55.33 ± 2.40	53.17 ± 4.31	56.67 ± 2.95
Mobility (second)	239.00 ± 17.03$^{\#\#}$	293.50 ± 11.83**,	338.83 ± 6.75**, $^{\#\#}$	334.17 ± 4.02**, $^{\#}$	332.17 ± 4.92**, $^{\#}$	348.83 ± 3.02**, $^{\#\#}$	350.5 ± 0.76**, $^{\#\#}$
Immobility (second)	121.67 ± 17.36$^{\#\#}$	66.50 ± 11.83**	21.16 ± 6.745**, $^{\#\#}$	25.83 ± 4.02**, $^{\#}$	27.83 ± 4.92**, $^{\#}$	11.16 ± 3.02**, $^{\#\#}$	9.50 ± 0.76**, $^{\#\#}$

Values are represented as Mean ± SEM, one way ANOVA followed by Dunnett's test for multiple comparisons

*P<0.05, **P<0.01 vs. normal control.

$^{\#}P$<0.05, $^{\#\#}P$<0.01 vs. standard.

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