



PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *PIPER CUBEB* LEAVES

¹Santosh M. Kurbetti*, Rakeshkumar Jat¹, Sachin Mali²

¹*Shri. Jagadish Prasad Jabarmal Tebriwala University, Jhunjhunu (Rajasthan)*

²*Y.D. Mane College of Pharmacy, Kagal, Kolhapur*

ABSTRACT

The Indian spices that provide flavor, color, and aroma to food also acquire many therapeutic properties. Ancient Indian books of Ayurveda, an Indian method of medicine, detailed the medicinal value of these plants and their therapeutic medicinal usage. Recently the scientific research has conventional the occurrence of many active compounds in these spices that are known to possess specific pharmacological medicinal properties. The therapeutic use of these being spices for definite pharmacological actions has also been conventional by experimental and clinical studies. The medicinal properties traditionally attributed to Indian spices are validated by modern pharmacological and experimental techniques, thus providing a scientific justification to their traditional therapeutic value. Many plant consequent molecules have shown a talented effect in therapeutics. Along with the plants investigated to date, one performance huge potential is the Piperaceae. Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family. In the present study, it was carried out to evaluate the *in vitro* antioxidant activity and preliminary phytochemical analysis of ethanolic, methanolic and aqueous leaf extracts of *Piper cubeba*. The *in vitro* antioxidant activity was evaluated by DPPH radical scavenging activity method. In *Piper cubeba* leaf the ethanolic extract showed antioxidant activity by inhibiting DPPH. Usually, antioxidant activity of ethanolic leaf extract of *Piper cubeba* may be due to the presence of tannins, saponins, phenols, flavonoids and alkaloids found in the preliminary phytochemical analysis.

Keywords: - Antioxidant activity, *Piper cubeba*, Phytochemicals, DPPH

INTRODUCTION

The antioxidants are substances that can prevent or slow injury to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers." A variety of herbs and components of foods or other ingestible substances that have potentially valuable effects on human health and purpose are used. In general, they are not being used as essential nutrients, but act as beneficial nutrients [1]. Many plant resulting molecule that have shown a capable effect in therapeutics [2]. Spices and herbs are accepted as sources of natural antioxidants and thus play a vital role in the chemoprevention of diseases and aging. Among the plants investigated to date, one performing huge potential is the pepper family otherwise known as Piperaceae [3]. *Piper cubeba* (black pepper) is flowering vine in the family Piperaceae. Superficially it will act as a rubefacient and as a local application for relaxed sore, throat and some skin disorder [4,5]. *Piper cubeba*, or tailed pepper, is a plant in genus Piper, cultivated for its fruit and essential oil. It is mostly developed in Java and Sumatra, hence sometimes called Java pepper. It is used to treat gonorrhoea, dysentery, syphilis, abdominal pain and asthma and has inhibitory effect on hepatitis C virus protease [6]. *Piper cubeba* was also shows an anti-inflammatory and analgesic activity of methanol extract from the fruit of *Piper cubeba* accumulates lignans and essential oil in a creatively high amount. The alkaloids, of which some 5,500 are known, consist of the major single class of secondary plant substance. Alkaloids are often toxic to man and many have dramatic physiological activities; therefore they wide use in medicine [7]. Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine) [8]. The dried cubeb berries contain essential oil consisting monoterpenes (sabinene 50%, α -thujene, and carene) and sesquiterpenes (caryophyllene, copaene, α - and β -cubebene, δ -cadinene, germacrene), the oxides 1,4- and 1,8-cineole and the alcohol cubebol. About 15% of a volatile oil is observed by distilling cubeb with water. Cubebene, the liquid portion, has the formula $C_{15}H_{24}$. It is a pale green or blue-yellow sticky liquid with a warm woody, slightly camphoraceous odor [9]. Unani doctors use a paste of the cubeb berries externally on male and female genitals to intensify sexual desire during coitus. Due to this credited property, cubeb was called "Habb-ul-Uruus"[10]. In the present study, an attempt was made to screen different multi solvent extracts prepared from dried Leaves of *Piper cubeba* for the study of antioxidant activity on basis of their phytochemical significance.

MATERIALS AND METHODS

Plant material:

The fresh leaves of *Piper cubeba* were collected from Sant Gajanan Maharaj Medicinal & Herbal garden, Mahagaon Site- Chinchewadi Taluka- Gadhinglaj Dist- Kolhapur (M.S.). These leaves are washed thoroughly in distilled water and the outside water was removed by air drying under shade.



Figure No. 1: *Piper cubeba* Leaves

Preparation of crude extract:-

The leaves were dried under shade condition and powdered mechanically. 100 gms of the dry powdered leaf samples of *Piper cubeba* was extracted for 8 hours with three different solvents like ethanol, methanol and aqueous by using soxhlet apparatus (Hot Extraction). The collected solutions were filtered through the Whatman No-1 filter paper. The extract were evaporated to dryness under reduced pressure at 90°C by Rotary evaporator and stored at 18 °C in a freeze until used for further tests.

Preparation of *Piper cubeba* Stock Solution:

Ethanolic, Methanolic and aqueous leaf extracts of *Piper cubeba* was prepared at the concentration of 1,000 µg/ml. From the stock solution different concentration viz. 20, 40, 60, 80, 100 and 120 µg/ml was prepared for antioxidant studies and ascorbic acid was used as standard for this study.

Qualitative Phytochemical analysis:

Freshly prepared extract was used for preliminary phytochemical screenings to find the presence of the phytoconstituent like; alkaloids, cardiac glycosides, Anthraquinones, Tannins, Phenols, Terpenoids, Steroids, Saponins and Flavonoids. A qualitative test was done by using standard procedures to identify the phyconstituents [11-14].

Phenolic estimation:-

Medicinal plants are a significant source of antioxidants. Natural antioxidants are the boost for antioxidant capacity of plasma and reduce the risk of convinced diseases. Polyphenols are the major plant compounds with antioxidant activity. Typical phenolics that have antioxidant activity are known to be largely phenolic acids and flavonoids. It is reported that the phenolics are responsible for the variation in the antioxidant action of the plant. They demonstrate antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [15]. The total phenol content of plant *Piper cubeba* extracts was determined by using Folin-Ciocalteu Spectrophotometric [16]. Reading samples on a UV-vis spectrophotometer at 650 nm. & the results was expressed as Catechol equivalents ($\mu\text{g}/\text{mg}$).

Antioxidative activity (DPPH Radical Scavenging Activity):-

The stable radical DPPH (1,1-Diphenyl-2-picrylhydrazyl) has been used broadly for the determination of primary antioxidant activity. The assessment or evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of (Blois, 1958) with some modifications [17]. Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 20, 40, 60, 80, 100 and 120 $\mu\text{g}/\text{ml}$ was added to 3 ml of a 0.004% ethanol solution of DPPH. An equal amount of ethanol and DPPH served as control. After 30minutes incubation in the dark, absorbance of the samples was measured at 517 nm. Radical scavenging activity was calculated using the following formula:

$$\text{Percentage radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100.$$

The antioxidant activity of the extract was expressed as IC₅₀ (Inhibitory Concentration 50). The IC₅₀ value was defined as the concentration (in $\mu\text{g}/\text{ml}$) of extracts that inhibits the formation of DPPH radicals by 50%. [18].

IC50 value (Inhibitory Concentration):-

IC50 value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. Results showed in Table-3 reports no IC50 value in water and methanol extraction of *Piper cubeba* plants only the ethanolic extract of the plants showed an IC50 value of $12.54 \pm 0.12 \mu\text{g}/\text{mg}$ that means ethanolic extract of *Piper cubeba* exhibited significant activity with low IC50. The antioxidant activity of *Piper cubeba* extracts increase with the increasing of polyphenol content of the extract. A linear relationship between the reciprocal of IC50 value and the total polyphenol content of *Pipercubebawas* observed in this study, indicating that increasing the polyphenol content strengthens the antioxidant activity.

RESULTS AND DISCUSSION

Qualitative Estimation of Phytochemical:-

Phytochemical analysis is most important for the evaluation of the qualitative analysis results, below is the discussion. This research work which was carried out on leaves of the medicinal plant *Piper cubeba* & showed the presence of a range of phytochemical constituents which was shown in Table-1.

In the present study, the attendance of high amount phytochemical like glycosides, alkaloids, tannins, flavanoids, phenols, polysterols polysaccharides and other all the principal secondary metabolites was detected in ethanolic extract of *Piper cubeba*. The living system is restricted from this by enzymes such as superoxide dismutase, glutathione peroxidase and catalase and certain endogenous antioxidant such as α -tocopherol, ascorbic acid, β -carotene and uric acid, since the endogenous antioxidants performing as intracellular safety systems defensive cells from free radicals damage and extensive lyses [19] Scavenging and retreating the formation of oxygen resulting species are not 100% efficient, micronutrients or antioxidants taken as supplements are chiefly significant in moving back the cumulative oxidative damages showed that biochemical basis enhanced drug availability by piperine. [20]. The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plant *Piper cubebastudied* showed that ethanolic extract of leaves of *Piper cubebais* rich in alkaloids, flavanoids, Glycoside, tannins, Polysaccharides

and Phenols. They were known to confirm medicinal activity as well as exhibiting physiological activity [21].

The effect of different solvents on the yields of crude extracts:-

There is some considerable variation in the yields of *Piper cubeba* extracts which was shown by using various fraction solvents. The yield of extracts using Water, Methanol and Ethanol of *Piper cubeba* are 4.20gm, 3.80gm and 4.70gm respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process Table-3.

Phenolic estimation:-

The total phenol content of plant *Piper cubeba* extracts was determined by using Folin-Ciocalteu Spectrophotometric. Reading samples on a UV-vis spectrophotometer at 650 nm. The results are expressed as Catechol equivalents ($\mu\text{g}/\text{mg}$). Phenolic content of *Piper cubeba* is 123.1 ± 0.05 ($\mu\text{g}/\text{g}$) Table-2.

DPPH scavenging activity

The constant radical DPPH has been used broadly for the determination of primary antioxidant activity. DPPH stable free radical method is an easy, rapid and sensitive way for the assessment of antioxidant activity of a specific compound or plant extracts. It is accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen donating capability [22]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neurodegenerative diseases.

Table-4 shows the results of the free radical DPPH scavenging activity in (%) inhibition. The result discovered that the ethanol fraction of *Piper cubeba* exhibited the maximum DPPH radical scavenging activity with $84.41 \pm 0.13\%$ at $120 \mu\text{g}/\text{ml}$ concentration (which is nearly close to the value of Ascorbic acid i.e. $88.65 \pm 0.26\%$) followed by $66.22 \pm 0.11\%$, $51.22 \pm 0.17\%$, $40.36 \pm 0.16\%$, $30.17 \pm 0.18\%$ and $19.10 \pm 0.13\%$ at the concentrations of $100 \mu\text{g}/\text{ml}$, $80 \mu\text{g}/\text{ml}$, $60 \mu\text{g}/\text{ml}$, $40 \mu\text{g}/\text{ml}$ and $20 \mu\text{g}/\text{ml}$ respectively. Similarly in case of methanolic extract the highest inhibition activity i.e. $76.17 \pm 0.26\%$ was found at $120 \mu\text{g}/\text{ml}$

followed by $61.10 \pm 0.14\%$, $47.34 \pm 0.11\%$, $33.07 \pm 0.12\%$, $23.26 \pm 0.17\%$ and $14.02 \pm 0.23\%$ at different range of concentration (100 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$, 60 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$) respectively. The order of percentage of scavenging activity in case of aqueous leaf extract of *Piper cubebawas* as follows: $73.11 \pm 0.18\%$, $60.31 \pm 0.19\%$, $45.21 \pm 0.36\%$, $34.36 \pm 0.31\%$, $23.22 \pm 0.11\%$ and $12.10 \pm 0.14\%$ at different concentration levels (120 $\mu\text{g/ml}$ -40 $\mu\text{g/ml}$) respectively.

The antioxidant capacity is also expressed as 50% inhibitory concentration (IC₅₀). A lower IC₅₀ value means a higher antioxidant capacity of the sample. Significantly lowest IC₅₀ value $12.54 \pm 0.12 \mu\text{g/ml}$ was observed in ethanolic extracts of *Piper cubeba* which is close to $12.68 \pm 0.14 \mu\text{g/ml}$ obtained in the standard ascorbic acid Table-3.

In generally the ethanolic leaf extract of *Piper cubeba* shows the highest scavenging activity followed by the aqueous and then methanol. Ethanol is chosen for the extraction of antioxidant compounds mostly because its lowers toxicity. It was observed that the antioxidant values were increased with increase in concentration of crude extracts which may be indicated that antioxidant values may be dependent on the occurrence of different phytochemicals such as alkaloids, flavonoids, saponins, tannins etc. It is reported that phenols are accountable for the variation in the antioxidant activity of the plant. It has been determined that the antioxidant effect of plant products is mostly due to radical scavenging activity of phenolic compounds such as alkaloids, flavonoids, phenols and tannins.

CONCLUSION

The present study results discovered that the ethanolic leaf extract of *Piper cubeba* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the leaves of *Piper cubebais* very much rich in different types of phytochemical constituents especially alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. So finally it can be concluded that ethanolic leaf extract of *Piper cubeba* can be used in pharmaceutical industries as natural antioxidants.

Acknowledgement

The authors are thankful to the Chairman Yeshwant Redekar College of Pharmacy, Principal Sant Gajanan Maharaj College of Pharmacy for providing necessary facilities for this research work.

Table No. 1: Preliminary Phytochemical screening of *Piper cubeba*. Leaves

Test	Plant Extract			
	Aqueous	Methanol	Ethanol	Chloroform
Alkaloid	+	+	+	+
Flavanoids	+	-	+	-
Glycosides	+	+	+	-
Reducing Sugar	-	-	-	-
Tannins	-	+	+	+
Saponins	-	-	-	-
Terpenoids	-	+	-	-
Polysaccharides	+	-	+	-
Phytosterols	-	-	+	-
Phenols	+	+	+	-

+: Present; -: Absent

Table No. 2: Phenol content of *Piper cubeba* Leaves in Ethanolic extracts.

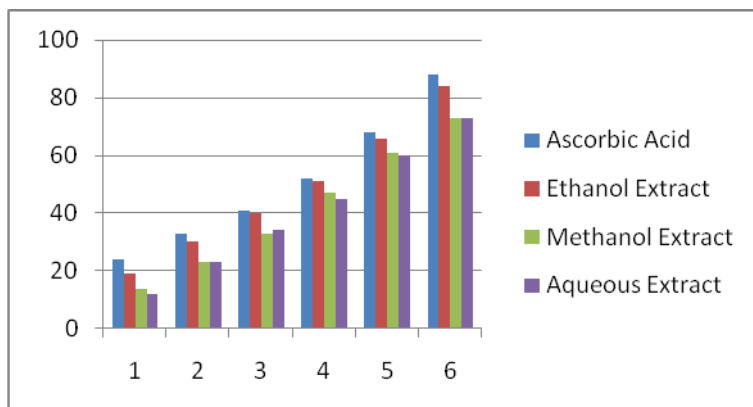
Solvent	Total phenols ($\mu\text{g/g}$)
Ethanol	123.1 \pm 0.05

Table No. 3: Crude extracts and IC₅₀ Values of *Piper cubeba* in different solvent extracts & ascorbic acid.

Solvent Used	Crude Extracts (gm)	IC ₅₀ Value ($\mu\text{g/ml}$)	Ascorbic Acid
Water	4.20	---	12.68 \pm 0.14
Methanol	3.80	---	
Ethanol	4.70	12.54 \pm 0.12	

Table No. 4: DPPH scavenging activity of *Piper cubeba* leaf extracted in different solvents

Concentration of extracts (µg/ml)	Antioxidant activity (%)			
	Ascorbic Acid	Ethanol Extract of <i>Piper cubeba</i> leaf	Methanol Extract of <i>Piper cubeba</i> leaf	Aqueous Extract of <i>Piper cubeba</i> leaf
20	24 ± 0.11	19.10 ± 0.13	14.02 ± 0.23	12.10 ± 0.14
40	33.19 ± 0.13	30.17 ± 0.18	23.26 ± 0.17	23.22 ± 0.11
60	41.37 ± 0.15	40.36 ± 0.16	33.07 ± 0.12	34.36 ± 0.31
80	52.29 ± 0.35	51.22 ± 0.17	47.34 ± 0.11	45.21 ± 0.36
100	68.11 ± 0.17	66.22 ± 0.11	61.10 ± 0.14	60.31 ± 0.19
120	88.65 ± 0.26	84.41 ± 0.13	76.17 ± 0.26	73.11 ± 0.18



Graphical Representation of DPPH scavenging activity of *Piper cubeba* leaf extracted in different solvents

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