Section A-Research Paper

COMPARATIVE ASSESSMENT OF ANTIOXIDANT POTENTIAL OF *PIPER CUBEBA* EXTRACT & ISOLATED COMPOUND

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Abstract

The discovery of novel bioactive phytochemicals is a key step in the possible production of new medications. The side effects of synthetic drugs lead us to recognize & isolate specific bioactive compounds with health benefits. Considering these recent developments this study specifically deals with analyzing the phytochemicals present in Root of *Panax ginseng*, flower of *Nymphaea stellata*, seeds of *Mucuna pruriens*, leaves of *Syzygiumcumini* and leaves of *Aegle marmelos.* The plant materials were collected & subjected to hydroalcoholic solvent for extraction. Further percentage yield & phytochemical studies were carried out on all the plant extracts. The results revealed that the percentage yield of different plant found to be vary slightly. In case of *Nymphaea stellate* & *Aegle marmelos* the extractive value was found to be7.80% & 7.51% respectively. The lowest yield of 6.14% is observed in case of *Mucuna pruriens*. For Panax *ginseng*the estimated yield came out to be 8.56%. The record of highest yield can be seen in case of *Syzygiumcumini.* The results of phytochemical study revealed the range of results. In case of *P. ginseng* extract almost all phytoconstituents were found to be present except alkaloid, tannin, proteins. Further *N.stellate* found to have components like alkaloid, flavonoid, diterpenes, Phenol & saponins. The *M. pruriens* also found to be laden with phytochemicals as all the test resulted positive except glycosides & tannins. The same was true with *S. cumini* as it is devoid of only alkaloid & Tannin. For *A. marmelos*extract alkaloid, flavonoid, phenol, protein, carbohydrate & saponin were found to be present. Thus, it can be concluded that all the studied plants have unique features & bioactive principles with therapeutic benefit.

Keywords: Herbal medicines, *Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium*cumini, *Aegle marmelos*, percentage yield, Phytochemicals

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Introduction

Free radicals are unfavourable substances that are created naturally by a number of biological processes in our bodies, including respiration, digestion, the metabolism of alcohol and drugs, and the conversion of lipids into energy. The structural change of cellular proteins and the changing of their activities are caused by excessive ROS generation, which causes cellular dysfunction and the disruption of essential cellular functions Lipid, protein, and DNA damage result from elevated ROS levels. The lipid membrane can be damaged by ROS, which can also enhance membrane fluidity and permeability. The protein damage includes proteolysis susceptibility, peptide chain breakage, cross-linked reaction product aggregation, electric charge change, and enzymatic inactivation. Last but not least, ROS can harm DNA by oxidising deoxyribose, causing strand breaks, deleting nucleotides, altering bases, and crosslinking DNA with proteins (Davies,2000; Sies et al., 2017; Dennery,2010).

The manifestation of oxidative stress can be influenced by the contemporary lifestyle, which includes an unhealthy diet, a lack of exercise, exposure to a variety of chemicals from many sources, including pesticides, heavy metals, food additives, and environmental pollution. According to various experimental and human research it may contribute to the rising burden of chronic illnesses. So, antioxidants may help to improve several chronic degenerative disorders in addition to helping to support healthy ageing (Halliwell,2007; Burton and Jauniaux,2011).

Reactive oxygen species (ROS) are chemical compounds that include superoxide radicals (O2•), hydrogen peroxide (H2O2), hydroxyl radicals (•OH), and singlet oxygen (1O2).A careful equilibrium between oxidants and antioxidants allows healthy organisms to resist the damaging effects of reactive oxygen species. Therefore, the steady creation of free radicals in aerobic organisms must be balanced by a constant consumption of antioxidants. Antioxidants, whether they are enzymatic or non-enzymatic, are chemicals that stop the generation of free radicals and work to neutralise or repair the harm they cause.Antioxidants stop these chain events by eliminating the free radical intermediates, and they also prevent additional oxidation processes by being oxidised. As a result, reducing agents like thiols, ascorbic acid, or polyphenols are frequently included in antioxidants.Animals and plants both have complicated systems of enzymes including catalase, superoxide dismutase, and different peroxidases, as well as antioxidants like glutathione, vitamin C, and vitamin E (Mattill*et al*., 1947; Vertuani*et al*., 2004; Leopoldini*et al*., 2011).

Piper cubeba L., a flowering creeper also known as Java Pepper, KabbabChini, or Cubeb pepper, is a member of the Piperaceae family and genus Piper. It is mostly grown for its fruit and volatile oil. Traditional uses for *P. cubeba* include the treatment of asthmatic illnesses,

gonorrhoea, dysentery, syphilis, stomach discomfort, diarrhoea, and enteritis (Abdul-Jalil and Nasser,2020; Andriana*et al*., 2019). The plant also has exceptional pharmacological properties. As an illustration, the essential oil of *P. cubeba* has antiparasitic, antibacterial, and insecticidal properties. Additionally, many plant extracts showed anti-inflammatory, nephroprotective, antioxidant, antibacterial, and hepatoprotective properties. These biological activities are due to its chemical composition, especially, phenolic acids and flavonoids, that have been detected in *P. cubeba* extracts. The plant is also a rich source of lignans particularly cubebin, a bioactive compound with a wide range of biological activities such as antimicrobial, anticaner, and neuroprotective, among others. Overall, the most reported modes of action by which *P. cubeba* extracts exert its biological activities involve many intracellular targets, among them the regulation of genes expression, inhibition of oxidative stress, induction of apoptosis and quorum sensing inhibition in pathogenic microbes (Kumar,2021; Drissi*et al*., 2022). Thus, reviewing its pharmacological activities this study aims at evaluating antioxidant potential of Pier cubeba extract & isolated compound extracted from same by using various antioxidant assays.

Materials & Methods

Collection of plant materials

Seed of *Piper cubeba*was collected from local market of Bhopal, M.P. The fresh seed parts of this species were washed under running tap water, shade dried at room temperature and powderedfollowing which they wereground to a fine powder, sieved through a 500-µm sieve,and stored until the extraction.

Extraction by maceration process

97 grams of shade dried powdered of*Piper cubeba* subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place. Defatted powder was measured and mixed with hydroalcoholic solvent (ethanol: water, 80:20v/v). This was left for 2 days in sterile environment. The liquid extract was then filtered through Whatman filter paper no.40. The filtrate was kept in water bath at 80-90ºC till the extract was dried out (Pandey and Tripathi,2014; singh*et al*., 2008).

DPPH method

The spectrophotometer was used to measure the DPPH scavenging activity. (Jain and Parkhe,2018). 1.5 ml of the stock solution (6 mg in 100 ml methanol) was made so that it produced an initial absorbance when combined with 1.5 ml of methanol. After 15 minutes, a decrease in absorbance was seen when sample extract at various concentrations (10-100 g/ml) was present. After diluting 1.5 ml of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading. In a series of volumetric flasks, 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added. The final volume was then adjusted to 3 ml with methanol. Three test samples were collected, and they were all handled similarly.

Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Nitric oxide method

The Griess reagent was measured after sodium nitroprusside was used to create nitric oxide. At physiological pH levels, sodium nitroprusside spontaneously generates nitric oxide in aqueous solution, where it interacts with oxygen to create nitric ions that may be measured using the Griess reagent. Nitric oxide production is reduced as a result of competition between nitric oxide scavengers and oxygen(Marcocci *et al.,* 1994). Different extract concentrations were combined with sodium nitroprusside (10 mmol/L) in phosphate buffer saline (PBS), and the mixture was then incubated at 25°C for 150 minutes. The specimens were treated with Griess reagent (1% sulphanilamide, 2% H3PO4, and 0.1% napthylethylenediamine dihydrochloride). The chromophore absorbance produced by the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was measured at 546 nm and referred to the absorption of ordinary ascorbic acid solutions treated in the same way with Griess reagent as a positive control.

Hydrogen peroxide method

Hydrogen peroxide was used to test the extract of *Piper cubeba*'s in-vitro antioxidant activity. Hydrogen peroxide (43 mol) in the amount of 2 ml was added, along with 1.0 ml of hydroalcoholic sample and 2.4 ml of 0.1 M phosphate buffer. (pH 7.4). After maintaining the resultant solution for 10 minutes, the absorbance at 230 nm was measured. Blank and control were both ready without the addition of hydrogen peroxide. It served as a typical component of an ascorbic acid complex. Calculated free radical hydrogen peroxide scavenging activity (percent) (Sroka and Cisowski,2003).

Results & Discussion

In plant biochemistry, the 2,2-diphenylpicrylhydrazyl (DPPH) test is frequently used to assess a plant's constituent's capacity to scavenge free radicals. The technique is based on spectrophotometric analysis of the change in DPPH concentration brought on by the interaction with an antioxidant. So, the antioxidant activity of *Piper cubeba* extract was first checked by DPPH method by using ascorbic acid as standard. Six different concentration from 10µg/ml to 100µg/ml was prepared. From the absorbance obtained IC 50 value was calculated. IC50 is the most popular and useful indicator of a drug's effectiveness. It provides a measurement of an antagonist medication's efficacy in pharmacological research by indicating the amount of drug required to block a biological process by 50%. So, the IC 50 value for ascorbic acid was observed to be 14.23 while that of hydroalcoholic extract was observed to be 225.74.

The same assay when performed with isolated compound from *Piper cubeba* extract it was seen that isolated compound have IC 50 value

as 23.45which is very appreciable when compared with standard.

Another method for analysing antioxidant the suppression of the nitric oxide radical produced by sodium nitroprusside in buffer saline. At 546 nm, the chromophore's absorbance is measured in the presence of scavengers. The activity is measured as a percentage decrease in nitric oxide. In this method concentration ranging from 20 µg/ml to Hydrogen peroxide scavenging assay was alternatively performed to check antioxidant potential of extract. The IC 50 value 37.96. The IC 50 value for extract & isolated compound

potential is nitric oxide method. This procedure is based on the Griess reagent's ability to detect

100 µg/ml was prepared. The IC 50 value for ascorbic acid estimated as 26.23. For hydroalcoholic extract it was observed to be 115.81 and for isolated compound it was recorded as 33.94. This indicates the isolated compound is more effective in reducing free radicals.

was found to be 121.96 & 56.16 respectively. So, from all the three assays results it can be concluded that isolated compoundhave far grater potential than extract as whole.

Table 1: % Inhibition of ascorbic acid and extract of *Piper cubeba*using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	41.93	3.83
2	20	56.45	5.64
3	40	61.29	7.67
4	60	72.58	13.76
5	80	75.8	18.28
6	100	80.64	23.25
IC_{50} value		**14.23**	**225.74**

Table 2: % Inhibition of ascorbic acid and isolated compound of *Piper cubeba*using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Isolated compound
1	10	41.93	37.51
2	20	56.45	52.67
3	40	61.29	59.84
4	60	72.58	67.96
5	80	75.8	72.36
6	100	80.64	77.49
IC_{50} value		**14.23**	**23.45**

Table 3: % Inhibition of ascorbic acid and extract of *Piper cubeba*using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	45.84	17.52
2	40	59.15	26.52
3	60	67.23	30.74
4	80	78.52	38.99
5	100	89.75	44.35
IC_{50} value		**26.23**	**115.81**

Table 4: % Inhibition of ascorbic acid and isolated compound of *Piper cubeba*using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Isolated compound
1	20	45.84	40.52
2	40	59.15	56.36
3	60	67.23	62.77
4	80	78.52	73.96
5	100	89.75	82.14
IC_{50} value		**26.23**	**33.94**

Table 5: % Inhibition of ascorbic acid and extract of *Piper cubeba*using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	40.51	9.47
2	40	51.96	16.51
3	60	62.47	25.16
4	80	64.22	33.87
5	100	67.84	40.96
IC_{50} value		**37.96**	**121.96**

Table 6: % Inhibition of ascorbic acid and isolated compound of *Piper cubeba*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Isolated compound
1	20	40.51	35.74
2	40	51.96	48.24
3	60	62.47	52.96
4	80	64.22	58.13
5	100	67.84	60.84
IC_{50} value		**37.96**	**56.16**

Conclusion

The current study on offers significant data that may be used in raw material quality control for authenticity and adulteration.It could be beneficial to swap out synthetic antioxidants for natural ones. The research of the plant's capacity to scavenge free radicals in the current study showed how effective it may be as a source of natural antioxidants. Therefore, more research should be conducted to evaluate its bioactivity using pure molecules in the creation of prospective pharmaceuticals. However, in the future, there will be more room for constituent separation, screening for antioxidant activity in separated constituents, and identification of active constituents using various spectrophotometric methods including UV-Visible, IR, NMR, and mass spectroscopy.The present study adds to the existing knowledge of *Piper cubeba* will be very useful for development of a formulation for treating various diseases.

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