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Phytochemical analysis and antioxidant capacity of black pepper extract

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Abstract
The king of spices our black pepper is blessed with immense usefulness and importance due to its medicinal properties, aroma and flavour. The micronutrient present in it gives it a highly antibacterial, antimicrobial, anti-inflammatory, anti-pyretic effect. The present scientific work depicts that black pepper was used to analyse TPC (total phenol content), TFC (total flavonoid content), antioxidant properties using DPPH assay. The current study, confirmed the presence of important secondary metabolites like, phenol, flavonoid, in the hydroalcoholic extract of black pepper. Total phenolic and flavonoid content of *Piper nigrum* was found to be 444.88 µg/g of tannic acid equivalent and 39.3049 µg/g QE equivalent.

Keywords: TPC, TFC, Phytchemcal, total carbohydrate

Introduction
Medicinal herbs provide remedies against diseases with higher efficacy. India has got varied climatic conditions which are favourable for growth of many species of plants. Our ancient texts like Vedas and bible provides us with the preparations techniques of natural products with curatives properties. Herbs and spices are considered an important part of human diet and have been used for thousands of years in traditional medicine and also to enhance colour, flavour and aroma of the food [Zarai *et al*., 2012] [1].
We use many types of herbs and its supplements in our diet which not only increases the flavour of our food but also boost our immunity and fights with the diseases. Black pepper is used in skin care, muscle and joint pains, and in improving blood circulation and respiratory systems. The bioactive molecule, piperine, present in pepper has major pharmacological impacts on the nervous and neuromuscular systems, exercises sedative effect and helps indigestion [Mukhopadhyay, 2000] [2]. The family Piperaceae comprises 12 genera and about 1400 species mainly found in tropical region. *Piper nigrum* L., sometimes called Indian Long Pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant, and has a similar, though generally hotter, taste [Ganesh *et al*, 2014] [3]. Our findings provides the information of phytochemical analysis suggesting different qualitative and quantitative estimation of alkaloids, flavanoids, carbohydrates, proteins etc. This study also investigates the utilization of black pepper for the synthesis of nanoparticle and tested for DPPH inhibitory activity of nanoparticle in herbal drug.

Materials and Methods
Sample Collection
100g Fresh pepper corn of *Piper nigrum* was collected from the local grocery shop of Bhopal, M.P. Black pepper was washed thoroughly with sterile distilled water and was crushed into powder.

Extraction
50g of powder was extracted using a mixture of 100ml Ethanol and 100ml distilled water in 1:1 ratio and was kept for three days at room temperature. Then filtered the extract and concentrate by vacuum evaporation.

Preliminary Phytochemical Analysis
Preliminary qualitative phytochemical screening were carried out for steroids (Salkowski test), terpenoids (Salkowski test), alkaloid (Wagner's Test), flavonoids (Lead acetate test), Tannins (Lead acetate test), Saponins (Forthing test), carbohydrates (Molisch's, Benedict's test), following the standard protocols (Minakshi *et al*., 2016) [4].

Quantitative test
Estimation of total phenol
Total phenolic content (TPC) was estimated according to the method of Ahmed *et al*., [2015] [9]. Plant sample was prepared by dissolving 4.3 mg in 10 mL methanol. The mixture was sonicated for 5 min to obtain a homogenized solution. To 300 µL of this solution taken in a test tube, 1 mL methanol, 3.16 mL distilled water and 200 µL Folin-Ciocalteu reagent were added. Then, after an 8 min incubation at room temperature, 600 µL sodium carbonate solution (10%) was added and the test tube was covered with aluminum foil and incubated in a hot water bath at 40 °C for 30 min. A blank was prepared using the same procedure, but replacing the plant extract with an equal volume of methanol. The absorbance of the sample was determined using a UV visible spectrophotometer at 765 nm. The standard curve of tannic acid was obtained using the same procedure. Total phenolic content was expressed as µg of tannic acid equivalents (TAE) per mL.

Total flavonoid content
The determination of total flavonoids was performed according to the colorimetric assay of Fuad Al-Rimawi *et al*., [2016] [6]. Distilled water (4 mL) was added to 1 mL of

the extract in a test tube. Then, 0.3 mL of 5% sodium nitrite solution was added, followed by 0.3 mL of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 5 minutes, and then 2 mL of 1 M sodium hydroxide was added to the mixture. Immediately, the volume of reaction mixture was made to 10 mL with distilled water. The mixture was thoroughly mixed using test tube shaker and the absorbance of the pink color developed was determined at 510 nm. Aqueous solutions of known Quercetin concentrations in the range of 50–100 mg/L were used for calibration and the results were expressed as mg Quercetin equivalents (QE)/g sample.

Total carbohydrate

The total carbohydrate was determined by phenol sulphuric acid method of Agrawal *et al*., (2015) [7]. A 0.2,0.4,0.6,0.8 and1ml of working standard (with 0.1mg/ml conc.) of glucose was taken in boiling tubes and the final volumes of each tube was made 1ml by adding distilled water. 1ml of 5% Phenol and 5ml of 96% Sulphuric acid was added one by one in each tubes and shook well so that the Phenol and Sulphuric acid get mixed thoroughly with working standard. After 10 minutes all the tubes were placed in water bath at 25-30°C for 15 minutes. Blank was set with 1ml of distilled water and O.D. of each tube was taken at 490nm with the help of spectrophotometer. Then the whole process following Phenol and Sulphuric acid method was repeated with 0.2ml of different samples of extract and the O.D.s of sample solutions were taken.

Results and Discussion

This study revealed various phytochemical constituents present in the plant extract which is depicted in the table1. Flavanoid, terpenoid, phenol, saponins and carbohydrates are present in it, which gives a good pharmacological effect and hence can be used in various pharmaceutical industries. Steroid was not found in the extract of black pepper.

Table 1: Qualitative estimation of phytochemicals is presented in the extract of black pepper

S. no.	Phytochemical test	Observations
1.	Alkaloid	Present
2.	Flavanoid	Present
3.	Terpenoids	Present
4.	Phenol	Present
5.	Steroids	Absent
6.	Saponnins	Present
7.	Carbohydrates	Present

Quantitative Estimation

Total phenolic content of *Piper nigrum* was found to be 444.88 µg/mg of tannic acid (graph 1.1) equivalent in hydroalcoholic extract. Phenolic contents are related to their antioxidant activity. Similar work was done by Andrade *et al* in 2013 and they found that 20.3 (mg GAE/g extract). Ahmad *et al* reported that total phenolic contents in methanolic extract were found to be 1.728 l mg/g.

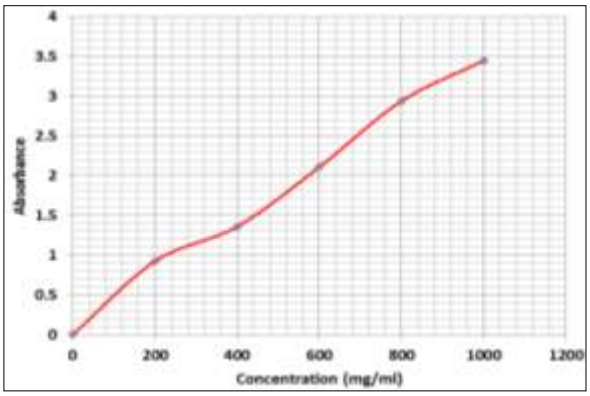

Fig 1: standard graph of tannic acid for total phenol content

The total flavanoid content of *Piper nigrum* was found to be 39.3049 QE equivalent in ethanolic extract. flavonoids provide stress protection, for example, acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction (Singh *et al*., 2012) [10]. Similar kind of work was done by Manzoor in (2012) and found that 39.564 ± 1.420 GAE/ gm of dried extract.

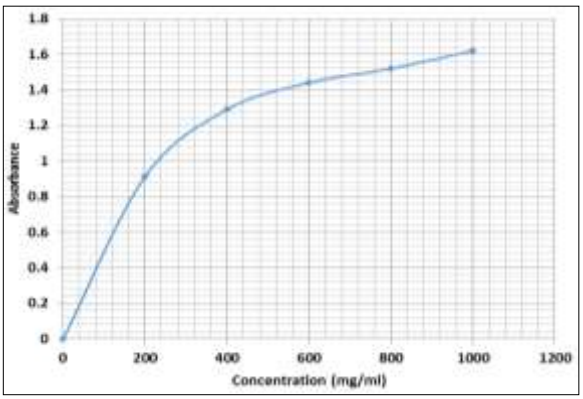

Fig 4: standard graph of tannic acid

Carbohydrate estimation *Piper nigrum* was found to be 305.1322 mg of glucose. A similar work was done in 2012 by Meghwal and Goswami and found Total carbohydrate (mg%) 43.2 at Panniyur (Kerala)

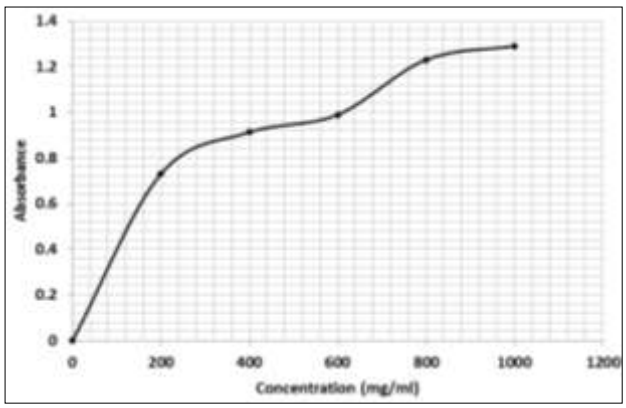

Fig 3: standard graph of glucose

Conclusion

Black pepper is a spice known for its pungent flavour and also for its beneficial properties to health. When comparing the different extraction methods, besides process yield, it is also necessary to estimate the antioxidant potential of the product by diverse procedures and also, in the future studies, evaluate the chemical composition of the extracts. Piperine is the major alkaloidal constituent of pepper. Systematic pharmacological studies on piperine have revealed its analgesic, antipyretic, anti-inflammatory and central nervous system depressant activities. It improves digestion by stimulating the taste buds in such a way that an impulse is sent to the stomach to increase hydrochloric acid secretion. these surmised all information on medicinal and nutritional value assessment of black pepper will end its utility in Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) Volume. 1 Issue 6, PP 24-27, 2015.

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