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ORIGINAL RESEARCH ARTICLE

Antioxidant screening of a polyherbal formulation composed of *Trigonella foenum-graecum* L., *Momordica charantia* L. & *Portulaca oleracea* L.

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ABSTRACT

Background: Traditional Medicines attained from medicinal plants are used by about 40-60 of the world's population. The present study concentrated on screening of polyherbal expression for *In Vitro* antioxidant activity.
Materials and Methods: Antioxidant activity of polyherbal formulation composed of *Trigonella foenum-graecum* L., *Momordica charantia* L. & *Portulaca oleracea* L. was performed by using DPPH and H_2O_2 assays.
Results: Polyherbal formulation (PHF) extracts displayed a marked antioxidant activity in DPPH and H_2O_2 assays. However, ethanol extract showed better results than n-hexane.
Conclusion: The present study successfully demonstrated the antioxidant activity of PHF in DPPH and H_2O_2 assays.

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INTRODUCTION

In the once many decades, there has been growing evidence that oxidative stress and specific human diseases can be prevented by including in the diet plant foods that contain large amounts of antioxidants similar as vitamins C, E or natural antioxidants such as flavonoids, tannins, coumarins, phenolics and terpenoids (Perumalla and Hettiarachchy, 2011).

Antioxidants are molecules that fight free radicals in our bodies. Free radicals are compounds that can cause harm if their levels become too high in our bodies. They are linked to multiple illnesses, including diabetes, heart disease, and cancer. Our body has its own antioxidant defenses to keep free radicals in check. Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical response that can produce free radicals, thereby leading to chain responses that may damage the cells of organisms (Ansari et al., 2010). Antioxidants similar to thiols or ascorbic acid (vitamin C) terminate these chain responses. To balance the oxidative stress, plants and animals maintain complex systems of lapping antioxidants, similar as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C and vitamin E (Kaliora et al., 2006).

Health conditions such as heart disease, macular degeneration, diabetes, and cancer are all influenced by cellular oxidative damage. There has been adding interest in the mechanism of action of antioxidants and whether they specifically block or remove free radicals from cells in the human body (Ames et al., 1993). Ames et al. (1993) reported that antioxidants prevent injury to blood vessel membranes, optimize blood inflow to

the heart and brain, prevent cancer-causing DNA damage, and lower the risks from cardiovascular and Alzheimer's conditions. Olinski et al. (2007) also indicated that antioxidants can prevent or slow the oxidative damage linked to colorful diseases such as carcinogenesis, atherogenesis, and aging. It's suggested that all these diseases might be retarded or prevented by protective compounds which have the ability to inhibit reactive oxygen species (ROS) formation, scavenge free radicals, or chelate metals (Faridi et al., 2008). In the body, natural endogenous antioxidant systems have been developed to deal with the product of free radicals and have been divided into enzymatic and non-enzymatic groups. Examples of the enzymatic antioxidants are superoxide dismutase, glutathione peroxidase, and catalase and non-enzymatic antioxidants are β-carotene, vitamin C, and vitamin E. There are also phytochemical antioxidants, such as polyphenols, lycopene, and lutein that can also protect the body from oxidation damage. Although there has been a focus on antioxidant effects of phytochemicals for numerous years, it's also recognized that-antioxidant effects similar to effects on cell signaling and gene expression are also important for health (Moon and Shibamoto, 2009).

Saponin glycosides, flavonoids, and alkaloids have been found in *Trigonella foenum-graecum* (fenugreek). Fiber, lipids, glycolipids, vitamins, choline, niacin, and other useful components are also abundant in fenugreek seeds. In streptozotocin-induced diabetic rats, *Trigonella foenum* seed (fenugreek) extract, alone and in combination, was found to ameliorate plasma glucose and cardiac antioxidant enzyme. Fenugreek seeds have been shown to have anti-cancer and antioxidant properties in some studies (Yadav and Baquer, 2014).

Momordicinin, cucurbitanes, terpenoids, and triterpenes are all found in *Momordica charantia* (bitter melon). Protein, sterols, fatty acids, and chemicals like menthol are also abundant. Minerals, vitamins, phosphorus, iron, carbohydrates, pantothenic acid, thiamine, riboflavin, niacin, and Vitamin B6 are all found in *Momordica charantia* pods. Several studies have found that a water-soluble extract of the fruit of *Momordica charantia* reduces the threat of diabetes.

Momordica charantia may promote the release of insulin when it's most demanded. It also helps to prevent dehydration, which is a common side effect of diabetes (Jia et al., 2017).

Purslane (*Portulaca oleracea*) is a largely nutritional plant that's high in omega-3 fatty acids, proteins, carbohydrates, and gum. It's high in vitamins A, B1, and C, as well as nicotinamide and a variety of minerals (magnesium, potassium, and nitrate). l-dopa, dopamine, and L-norepinephrine, as well as two red-violet betacyanins (operation I and II), caffeic, ferulic, and sinapic acid, calcium oxalate and oxalic acid, potassium salts, and iron saccharate, are all found in the plant (Rahimi et al., 2019).

The goal of the present study was to assess the antioxidant capacity of a polyherbal formulation (*Trigonella foenum-graecum* L., *Momordica charantia* L., and *Portulaca oleracea* L.) using in vitro assays.

MATERIALS AND METHODS

Plant material

Trigonella foenum-graecum L., *Momordica charantia* L., and *Portulaca oleracea* L. are the main ingredients in this polyherbal mixture. A taxonomist authenticated the plant materials gathered, and samples were lodged with the Department.

Chemicals and equipment

Ethanol, Petroleum ether,-Diphenyl-2-picrylhydrazyl (DPPH), BHA (butylated hydroxyanisole), ascorbic acid, phosphate buffer, H_2O_2 was acquired from Sisco Research Laboratories Pvt. Ltd. and S.D. Fine Chem. Ltd. India, UV-Visible Spectrophotometer.

Preparation of plant extract

The 1000g polyherbal formulation was defatted with petroleum ether before being extracted with ethanol using the soxhlet equipment. Following the conclusion of each solvent's extraction, filtering and concentration were performed. Each solvent extract was weighed and kept once the solvent had fully evaporated.

Table 1. Physical characteristic features of ethanol and n-hexane extracts of PHF.

S. N.	Extract	Colour	Consistency	Percentage Yield (% w/w)
1.	Ethanol	Light cream	semi solid	14.28
2.	n-hexane	Dark green	Solid	13.22

Qualitative phytochemical analysis

According to Khandelwal (2002), the test samples were subjected to phytochemical analysis to determine the existence of phytochemical contents, and the phytochemical constituents were detected (Khandelwal, 2002).

In Vitro antioxidant activity assays

In vitro studies are performed with microorganisms, cells, or biological molecules outside their normal biological context. Colloquially called" test- tube experiments", these studies in biology and itssub-disciplines are traditionally done in labware alike as test tubes, flasks, Petri dishes, and microtiter plates (Blois, 1958; Desmarchelier et al. 1997).

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) assay

The antioxidant activity of extracts of the PHF plant was determined using the 1, 1-diphenyl-2 picryl hydrazyl (DPPH) free radical scavenging assay (Blois, 1958; Desmarchelier etal. 1997). DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in color (from deep violet to light yellow) was measured. The intensity of the yellow color depends on the amount and nature of radical scavengers present in the sample or standard compounds (Blois, 1958; Desmarchelier etal. 1997).

DPPH assay of PHF was performed as per the method of Blois (1958) and Desmarchelier etal. (1997). The two different yields of PHF were mixed with 95 methanol to prepare the stock solution in the required concentration (10 mg/ 100 ml or 100 µg/ ml). From the stock solution 1 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml of this solution were taken in five test tubes and by serial solution with the same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 10 µg/ ml, 20 µg/ ml, 40 µg/ ml, 60 µg/ ml, 80 µg/ ml and 100 µg/ ml respectively. Ascorbic acid was used as a standard was prepared in the same concentration as that of the sample extract by using ethanol as solvent. The final reaction mixture containing 1 ml of0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Decrease in the absorbance in the presence of sample extract at different concentrations of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg/ ml) was noted after 15 min incubation period at 370C. Methanol (1.0 ml) plus PHF solution (2.5 ml) was used as a blank. A decrease in absorbance is the presence of sample extract, and standard at different concentrations was noted after Absorbance was read out at 517 nm using a double- rayU.V. Spectrophotometer (SHIMADZU UV-1700).

Inhibition = (A Blank – A Test)/ A Blank × 10

Where A is Absorbance.

Hydrogen peroxide scavenging (H_2O_2) assay

Human beings are exposed to H_2O_2 indirectly via the environment nearly about0.28 mg/ kg/ day with intake substantially from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage in the body. The ability of plant extracts to scavenge hydrogen peroxide can be estimated (Blois, 1958; Desmarchelier etal. 1997).

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. All the two extracts of the PHF in different concentrations were added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

Scavenged (H_2O_2) = [(Ai – At)/ Ai]× 10

Where Ai is the absorbance of the control and At is the absorbance of the test.

R E S U L T S

Qualitative phytochemical analysis

Extracts were subjected to qualitative evaluation in order to find out the chemical constituents present. The following phytoconstituents were present (Table 2).

Table 2. Comparative phytochemical composition of ethanolic and n-hexane extracts of PHF.

Phytochemical constituents	Ethanol extract of PHF	n-hexane extract of PHF
Alkaloids	+ve	+ve
Carbohydrates	+ve	+ve
Glycosides	+ve	+ve
Protein	+ve	+ve
Tannins	+ve	+ve
Flavonoids	+ve	+ve
Saponin	-ve	+ve

In Vitro antioxidant activity of the PHF extracts

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) assay

Results obtained for in vitro inhibition of DPPH (-Diphenyl-2 picryl hydrazyl) assay are tabulated in Figure 1 & 2. All the two excerpts were screened for DPPH radical scavenging activity with percentage inhibition ranges from31.28 to83.96 in which the highest activity was detected in Hydro ethanolic extract (IC 50 value91.92 µg/ ml) followed by n-hexane extract (IC 50 value µg/ ml respectively). The t- test analysis showed that there's a significant difference in the DPPH radical scavenging activity among the different extracts of the test sample and standard ascorbic acid with (IC 50 value7.089 µg/ ml).

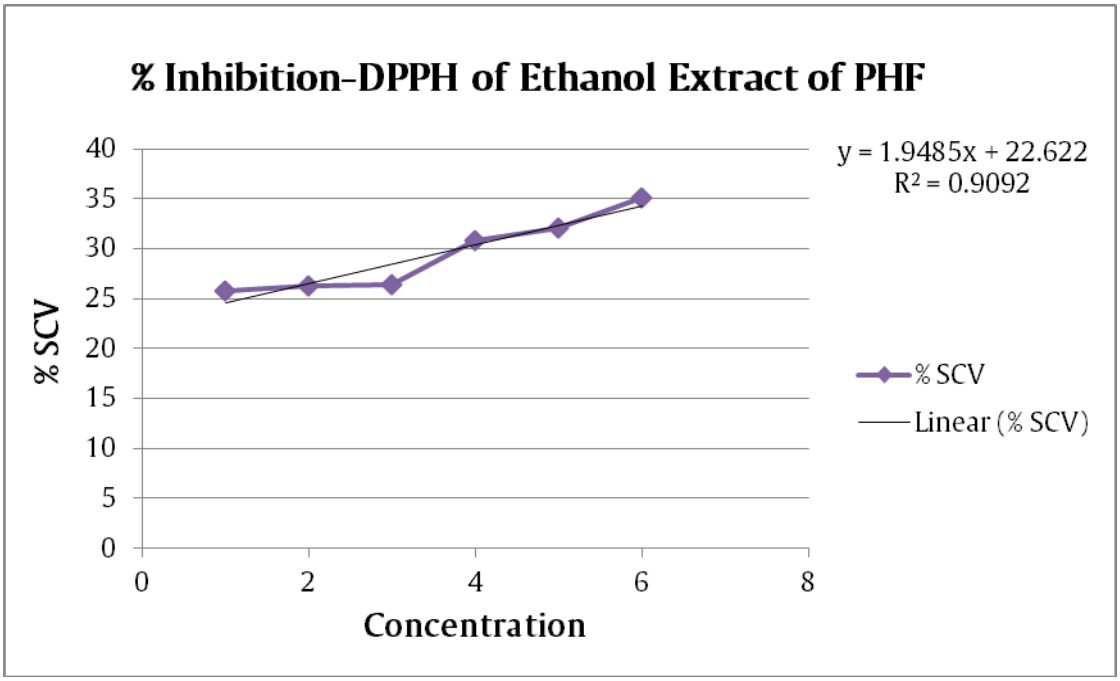

Figure 1. Percentage inhibition of ethanol extract of PHF on DPPH radical scavenging assay.

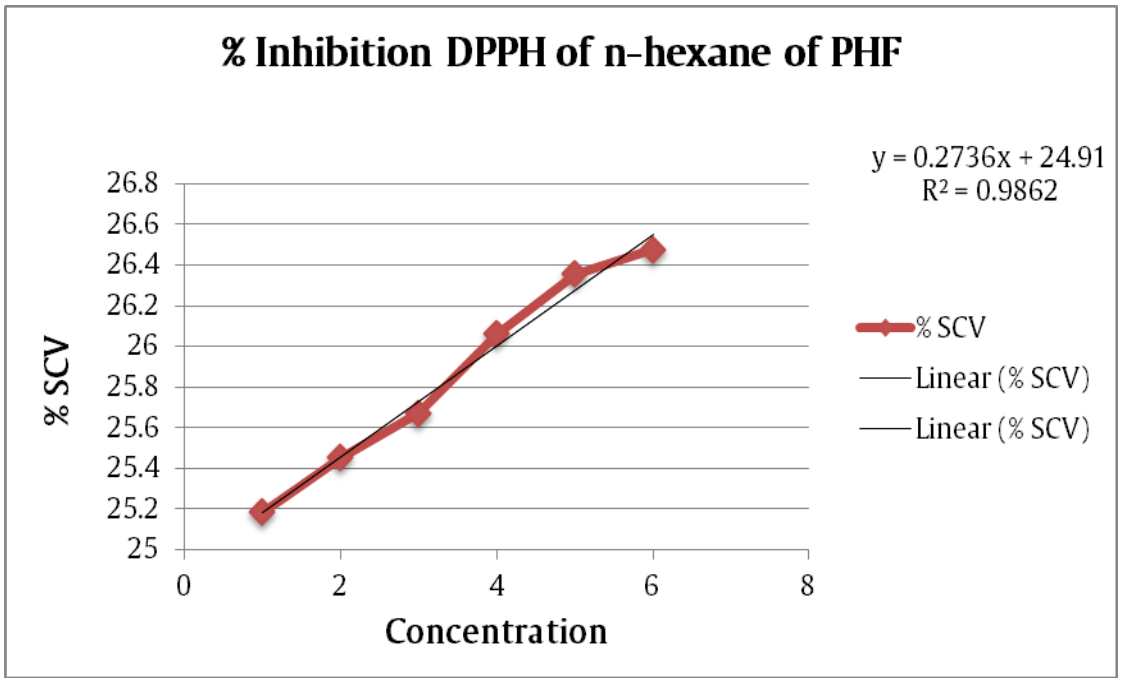

Figure 2. Percentage inhibition of n-hexane extract of PHF on DPPH radical scavenging assay.

Hydrogen peroxide scavenging (H_2O_2) assay

All the two extracts were screened for H_2O_2 radical scavenging activity in which the highest activity was detected in ethanolic extract (IC 50 value29.52 µg/ ml) followed by n-hexane extract (IC 50 value 0.639 µg/ ml respectively). The t- test analysis showed that there's a significant difference in the H_2O_2 radical scavenging activity among the different extracts of the test sample and standard.

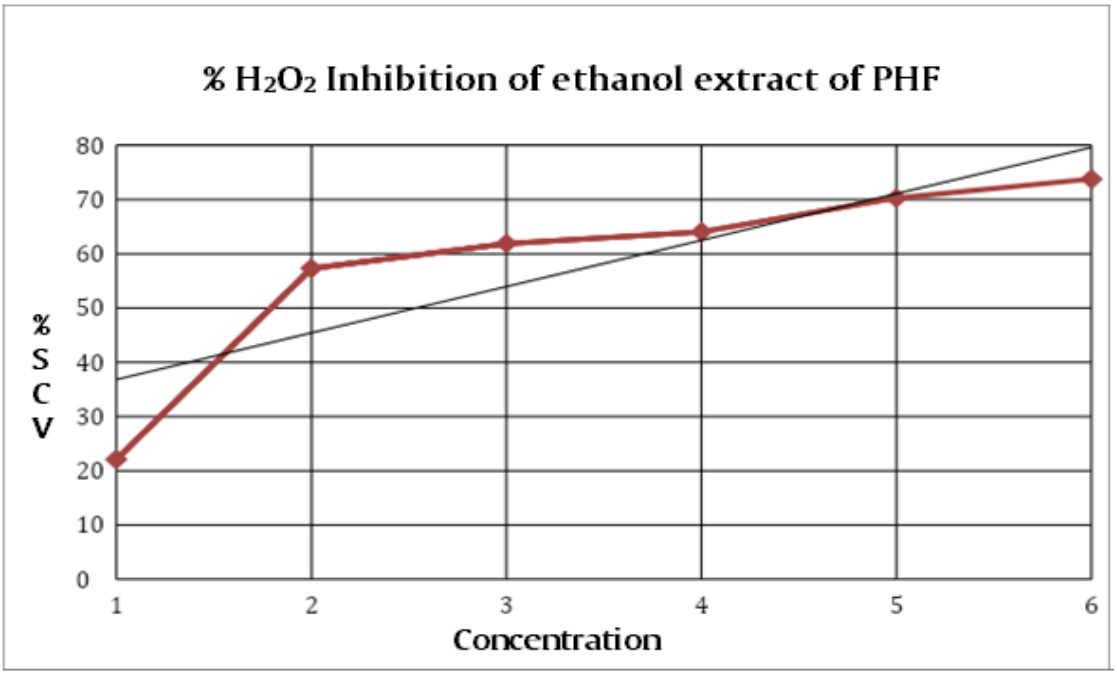

Figure 3. Percentage inhibition of ethanol extract of PHF on H_2O_2 radical scavenging assay.

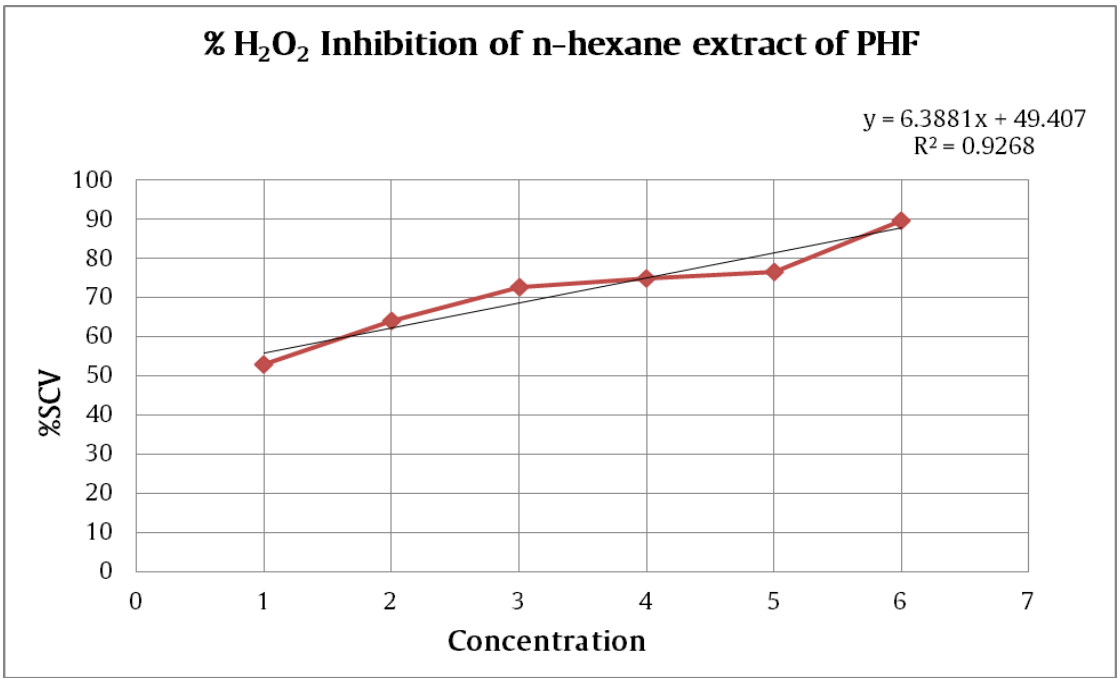

Figure 4. Percentage inhibition of n-hexane extract of PHF on H_2O_2 radical scavenging assay.

DISCUSSION

Oxidative stress is an imbalance between free radicals and antioxidants in your body. Free radicals are oxygen- containing molecules with an uneven number of electrons. The uneven number allows them to easily react with other molecules. Free radicals can cause large chain chemical reactions in your body because they reply so easily with other molecules. These reactions are called oxidation. They can be beneficial or harmful (Koleva et al., 2002).

Comparative % Inhibition of (DPPH Assay) of Both Extracts of PHF.

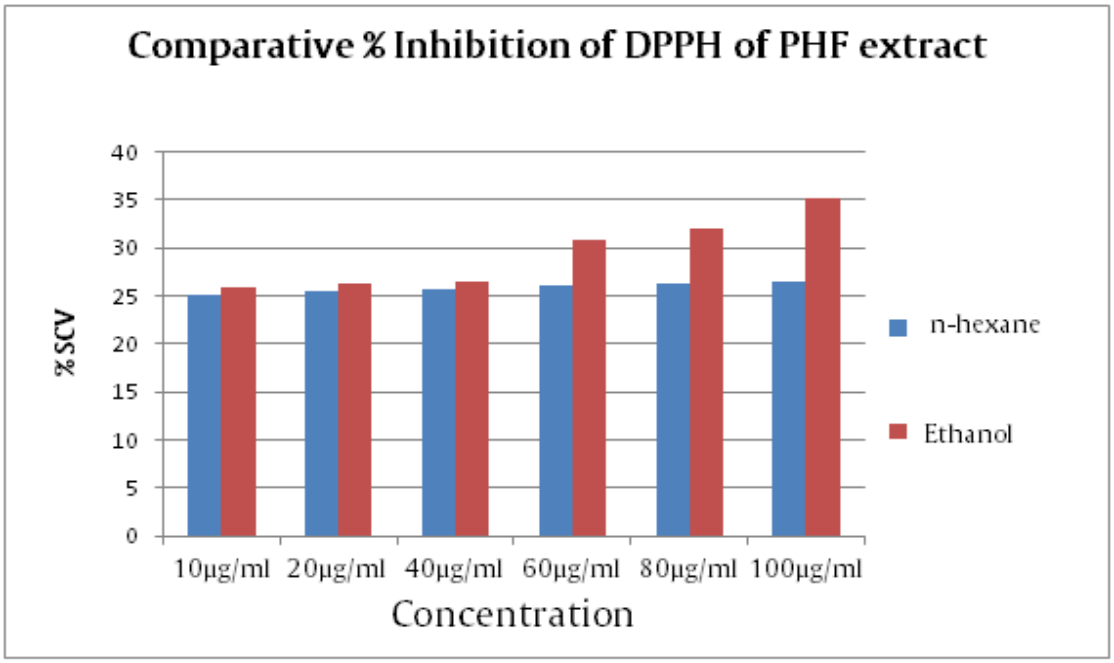

Figure 5. Antioxidant Activity (DPPH scavenging assay) of both extracts of PHF.

Comparative % inhibition of (H_2O_2 scavenging assay) of both extracts of PHF.

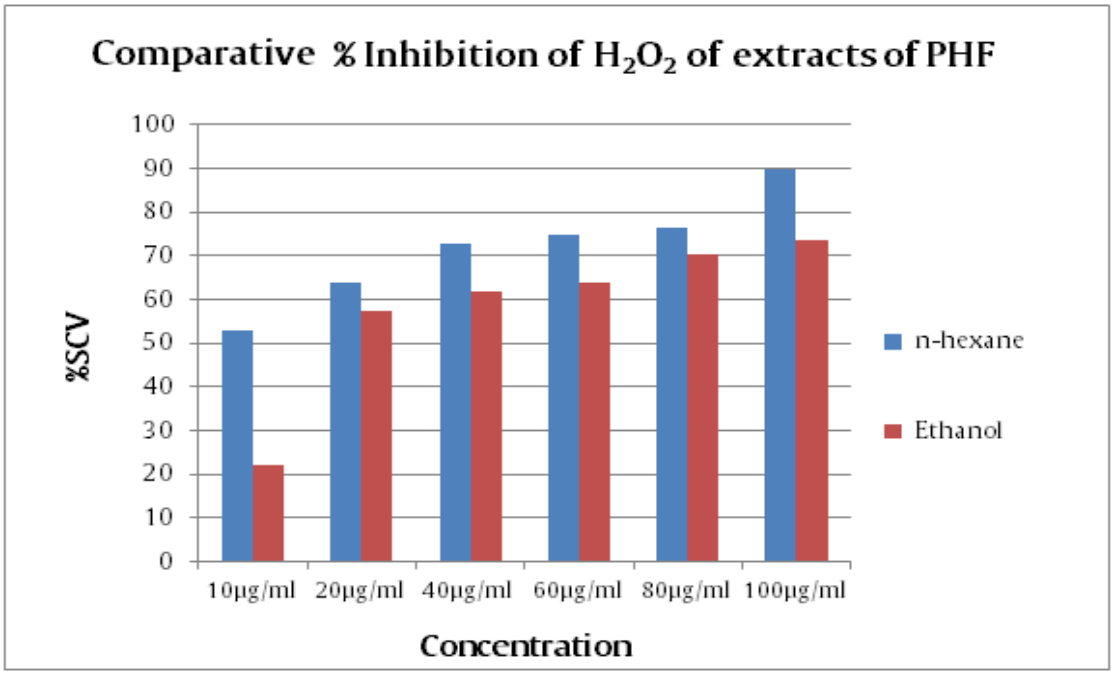

Figure 6. Comparative antioxidant activity (H_2O_2 scavenging assay) of extracts of PHF.

The present research work was aimed to study the antioxidant activity of PHF using *In Vitro* screening models. The quantification of total alkaloids, total saponins, and total polyphenols revealed that the polyphenols were present in the greatest proportion.

PHF has considerable antioxidant activity. These results justify traditional medicine use as a safe herbal remedy to protect from the free radicals that can start doing damage to fatty tissue, DNA, and proteins in the body so that damage can lead to a vast number of diseases over time and to treat oxidative stress (Koleva et al., 2002; Mathiesen et al., 1995).

Qualitative chemical investigation of both the extracts PHF dried revealed the presence of certain important phytoconstituents such as glycosides, saponins,

flavonoids, and phenolic compounds predominantly in the non-polar extracts (Table 1 & 2).

A number of researchers have worked on the isolation and identification of natural antioxidants from medicinal plants. Ahmad et al. (2010) reported the antioxidant activity of *Pistacia integerrima* leaf extract. Eshwarappa et al. (2014) determined the antioxidant activities of aqueous and ethanolic *Syzygium cumini* leaf gall extracts using DPPH and FRAP methods. According to their findings, the alcoholic extract was 30% more potent than the aqueous extract (Ahmad et al., 2010; Eshwarappa et al., 2014).

The antioxidant activities of all the prepared extracts were measured. In this way, serial dilutions (10-100 µg/ml) of each extract or fraction were measured to obtain EC50 (the amount of compound that gives half-maximal response). The DPPH radical scavenging activity of all fractions steadily increased in a concentration-dependent manner. Effectiveness in reducing power was in descending order from ethanolic extract of PHF to n-hexane extract of PHF (Figure 1 & 2). The ethanolic extract of PHF gives antioxidant scavenging DPPH potential slightly less than that of ascorbic acid, the standard powerful antioxidant.

As the absorption at 517 nm decreased, the radical scavenging activity increased. The antioxidant activity of different extracts showed that the highest antioxidant potential was with ethanol followed by n-hexane fraction which may be due to its containing of phenolic and flavonoid compounds (Figure 3 and 4). The antioxidant activities of all the prepared extracts were measured. In this way, serial dilutions (10-100 µg/ml) of each extract or fraction were measured to obtain IC50 (the amount of compound that gives half-maximal response). The results were listed in Figure 3 and 4. The H_2O_2 scavenging activity of all fractions gradually increased in a concentration-dependent manner. Effectiveness in reducing power was in descending order: ethanolic extract of PHF > n-hexane extract of PHF (Figure 5 and 6). The ethanolic extract of PHF showed antioxidant scavenging H_2O_2 potential slightly less than that of the standard powerful antioxidant. As the absorption at 230 nm decreased, the radical scavenging activity increased.

The antioxidant activity of different extracts showed that the highest antioxidant activity was in ethanol fraction followed by an n-Hexane fraction which may be due to its containing of phenolic and flavonoid compounds (Figure 5 and 6).

CONCLUSION

It is concluded that PHF has potent antioxidant activity in all assays tested due to its oxidative stress effects. The antioxidant activities of two extracts of PHF were examined for the radical-scavenging activity of DPPH and H_2O_2 scavenging assay. Phytochemical screening of PHF using *In Vitro* techniques for detecting antioxidant property provided a scientific basis and justification for its use. This opens a new dimension and rationale for the use of PHF in the modern system of medicines. It can be used as a drug or herbal supplement in antioxidant therapy after thorough research.

CONFLICT OF INTEREST

None declared

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