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Total phenolic, flavonoid content and hepatoprotective potentials of *Peltophorum pterocarpum* (DC) K Heyne leaf extracts

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Abstract

Herbal drugs are traditionally used in various parts of world to cure different diseases. In the present research studies, *Peltophorum pterocarpum* (DC) K Heyne was used as herbal drug and its hepatoprotactive effect in CCl_4 induced liver injury in mice was evaluated, using essential biochemical parameters like total bilirubin, SGOT, SGPT and ALP. In this experiment, pretreatment of mice with *Peltophorum pterocarpum* leaf extracts, resulted in significant reduction in all the liver marker enzymes and bilirubin. Total phenolic and flavonoid contents were evaluated according to Folin-Ciocalteau procedure and a calorimetric method, respectively. It showed high content of total phenolic compounds, total flavonoids and polyphenolic compounds.

Key words: *Peltophorum pterocarpum* (DC) K Heyne, Total phenolics, Flavonoid, Extraction, Hepatoprotective activity

Introduction

Liver is the largest organ in the human body and key organ of metabolism, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification (Opoku *et al*., 2007). Liver disease is still a world wide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli, 2006). It is continuously and variedly exposed to xenobiotics, environmental pollutants, and chemotherapeutic agents because of its strategic placement in the body (Ibrahim and Khaja, 2008). If the natural protective mechanisms of the liver are overpowered during all such exposures, this will lead to hepatic injury. In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda, recommended for the treatment of liver disorders (Chatterjee, 2000). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube *et al*., 2008). Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values (Marimuthu *et al*., 2008). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggest that, in order to find active compounds, a systematic study of medicinal plant is very important (Canadanovic-Brunet *et al*., 2005). *Peltophorum pterocarpum* (DC) K Heyne (Fabaceae) is commonly called as copper pod, yellow poinicana. Its Indian name is Radhachura. *Peltophorum pterocarpum* (DC) K Heyne leaves have been reported to exhibit antibacterial and antifungal effects. Leaves are also reported to possess antiulcer and anti-inflammatory activity (Sethuraman *et al*., 1984). The aim of the present study was to estimate total phenolic, total flavonoid content and to determine hepatoprotective effect of *Peltophorum pterocarpum* (DC) K Heyne.

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Materials and Methods

Extraction of plant material

The fresh leaves of *Peltophorum pterocarpum* (*DC*) K Heyne (Fabaceae) were collected from Gulbarga University

Campus, Gulbarga (Karnataka) India in the month of April-May 2009. The plant was identified and authenticated (Department of Botany HGUG No.47). The shade dried leaves of *Peltophorum pterocarpum* (DC) K Heyne were powdered to 22 mesh size, using the electric blender and then subjected to successive Soxhlet extraction with petroleum ether (40° - 60°C), chloroform, methanol and distilled water until the solvents became colourless. The extracts, thus, obtained were further evaporated to dryness under vacuum and refrigerated for future use.

Extraction of polyphenols

Finely powdered leaf sample (500 gms) was mixed with 70% ethanol and kept at room temperature for 5 days. After 5 days, solution was filtered and solvent was evaporated. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/ 1ml). Extraction of polyphenols was carried out for 36 hours at room temperature then the aqueous layer was discarded; combined ethyl acetate layer was concentrated which contained polyphenols (Xia *et al*., 1998).

Estimation of total phenolics

1ml of extract solution containing 1g extract in the volumetric flask was diluted with 45 ml of distilled water. 1ml of Folin-Ciocalteau reagent was added and the content of flask was mixed thoroughly. 3 minutes later, 3ml of 2% saturated sodium carbonate was added to the mixture and was allowed to stand for 2 hours with intermittent shaking. The absorbance was read at 760 nm. The concentration of total phenolics was expressed as mg/g of dry extract (Malic and Singh 1980). The concentration of total phenolics in extract was determined as µg of gallic acid equivalent using an equation obtained from standard gallic acid curve.

$$\text{Absorbance} = 0.0008 \times \text{gallic acid (µg)}$$

Estimation of flavonoids

1g of sample was added to 1ml of 80% ethanol. Aliquot of 0.5 ml was added to the test tube containing 0.1ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 minutes of incubation at room temperature. Total flavonoid concentration was calculated using rutin calibration curve (Helmja *et al*., 2007).

Hepatoprotective studies

Male Swiss albino mice of 30-35 g were divided into four groups, containing six animals in each. The first group which served as control was administered with saline, the second group (negative control) was administered with carbon tetrachloride 50 µl/kg (1:1 v/v). The third and fourth groups were served with methanolic (1g/kg) and polyphenolic (100mg/kg) extracts, respectively for 10 days via oral route. The dose was selected on the basis of the LD_{50}. On 11th day, blood samples of animals were used for hepatoprotective studies. Different parameters such as SGOT, SGPT, ALP and total bilirubin content of the serum were analyzed by using commercial kits (Accurex Biomedical Pvt. Ltd. Mumbai, India) (Kotamballi *et al*., 2002).

Data analysis

Arithmetic mean and standard error of mean were calculated from the individual observation. The data are expressed as Mean ± $SE_{\bar{x}}$. Statistically difference between mean were calculated, using one-way-analysis of variants, followed by Tukry-Kramer 't'. $p < 0.001$ was considered statistically significant.

Results and Discussion

Phytochemical screening of the methanolic extract revealed the presence of phenolics, flavonoids, saponins, tannins *etc*. Table 1 represents the total phenols and flavonoid content of the *Peltophorum pterocarpum* (DC) K Heyne leaves. Total phenols of methanolic and polyphenolic extract were found to be 242 mg/g and 120 mg/g, respectively, whereas flavonoids were found to be 72 mg/g in methanolic extract and 38.5 mg/g in polyphenolic extract.

Phenolic compounds are commonly found in plant kingdom and they have been reported to have many biological effects. Correlation between the contents of phenolic compounds and antioxidant activity has been described in many studies. Similarly the ability of flavonoids (polyphenols) to act as antioxidants has been extensively investigated (Helmja *et al*., 2007). Presence of phenols and flavonoids in methanolic and polyphenolic extracts of the leaves in higher concentration suggest that they could be better antioxidants.

Hepatoprotective studies

The liver damage in carbon tetrachloride induced hepatotoxicity is mainly assessed by determining the serum enzyme levels. Liver is considered to be highly sensitive to toxic agents. Carbon tetrachloride administration causes membrane damage of liver, thereby, releasing enzymes into the blood circulation which can be determined by using serum sample or blood sample. Figure 1 represents the bilirubin content, SGOT, SGPT and ALP. It was observed that the animals treated with carbontetrachloride, resulted in the significant hepatic damage as shown by the elevated levels of marker enzyme. The treatment with *Peltophorum pterocarpum* (DC) K Heyne methanolic and polyphenolic extracts significantly attenuated the elevated levels of serum markers. The normalization of serum markers by the extract suggests that they are able to condition hepatocytes as to protect the membrane integrity against carbon tetrachloride induced leakage of marker enzyme in the circulation. The above changes can be considered as an expression of functional

improvement of hepatocytes which may be caused by accelerated regenerations of parenchyma cells (Deepak *et al*., 2007). Carbontetrachloride has been extensively studied as a liver toxicant and its metabolites such as trichloromethyl radicals (CCl_3) and trichloromethyl preoxy radical (CCl_3O_2) are involved in the pathogesis of liver (Singh *et al*., 2002).

The effect of free radical on liver detoxificant enzymes reduces the enzyme activity, due to enzyme inactivation during the catalytic cycle. The *Peltophorum pterocarpum* methanolic and polyphenolic extracts contained flavonoid, polyphenol; catechol, *etc*. which might act as potent free radical scavengers, redcing the levels of hydrogen peroxide (H_2O_2) and superoxide ions consequently. This may also point towards the possible do novo syntehsis of these enzymes, induced by the components of *Peltophorum pterocarpum* (Aroumc, 1994).

Conclusion

The present investigation demonstrates total phenolic, flavonoid content and hepatoprotective potentials of *Peltophorum pterocarpum* (DC) K Heyne leaf extracts. Pretreatment of mice with *Peltophorum pterocarpum* (DC) K Heyne extract resulted in significant reduction in total bilirubin content, SGOT, SGPT and ALP. This study, thus, indicates that the extracts obtained from the leaves of *Peltophorum pterocarpum* (DC) K Heyne can be a potential source of natural medicine for liver protection.

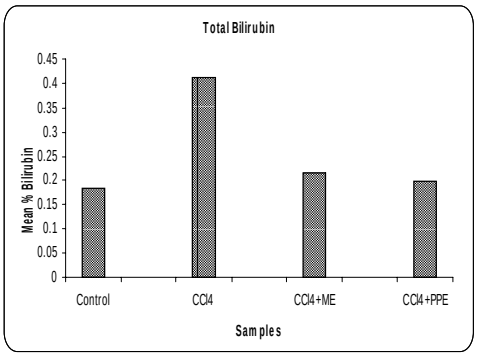
Table 1: Total phenolic and flavonoid content of *Peltophorum pterocarpum* (DC) K Heyne leaf extracts

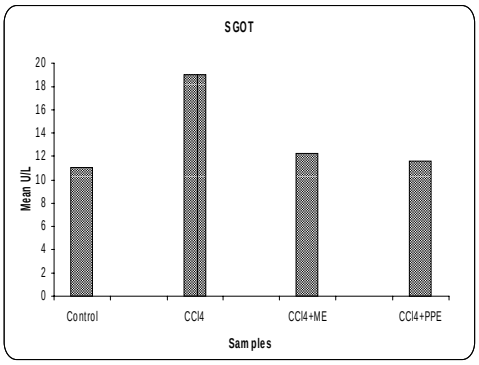
Test Sample (1gm/ml)	Total phenols (mg/g)± $SE_{\bar{x}}$	Total flavonoids (mg/g)± $SE_{\bar{x}}$
Methanol extract	242±0.012	72.1±0.002
Polyphenolic extract	120±0.001	38.5±0.002

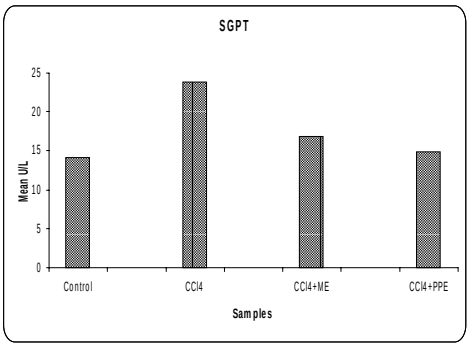
Total phenols are expressed as catechol equivalent per gram. Total flavonoids are expressed as rutin equivalent per grams

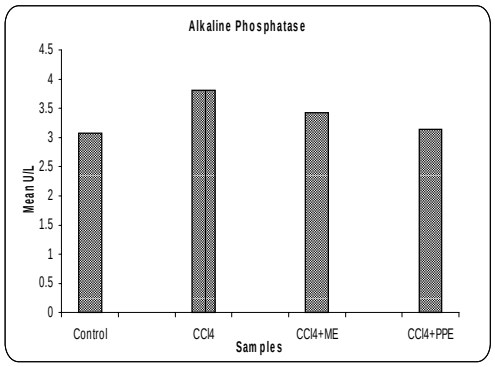
$SE_{\bar{x}}$ - Standard error of mean

Figure 1: Hepatoprotective activity of *Peltophorum pterocarpum* (DC) K Heyne leaf extracts on total bilirubin, SGOT, SGPT and ALP against carbon tetrachloride induced toxicity (ME- Methanolic Extract, PPE- Polyphenolic Extract)









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