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Research Article

INORGANIC STATUS OF STEM BARK OF *PTEROCARPUS MARSUPIUM*

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ABSTRACT
Pterocarpus marsupium is well known for its sugar lowering potential. In the present examination different bark samples (Apical bark, middle bark and mature inner bark) of *Pterocarpus marsupium* were screened for inorganic status. The levels of macro-minerals Nitrogen (1.50-3.13%), Phosphorus (0.023-0.163%), Calcium (0.60-1.848%), and Magnesium (0.21-0.339%), levels of trace minerals Copper (0.68-3.2mg/100g), Zinc (1.98-3.62mg/100g), Manganese (2.0-4.94mg/100g) and Iron (11.38-44.34mg/100g) and heavy metals Chromium (2.08-3.94mg/100g) and Nickel (0.32-1.26mg/100g) were evaluated in the present study. Cadmium and Lead were found to be absent in all the bark samples analyzed.
KEY WORDS: *Pterocarpus marsupium,* Inorganic analysis, macro-minerals, traces minerals, heavy metals.

INTRODUCTION

Pterocarpus marsupium, member of family fabaceae, is commonly known as Indian Kino. The bark exudes a red gummy substance called 'Gum Kino' when injured. Ethno-botanically the powdered bark is mixed with *Schleichera oleosa* and taken with cold water to treat dysentery[1]. Tribal people residing in the Jodhalal forest of Karnataka use stem bark to treat the wounds, fever, stomachache, diabetes and elephantiasis[2]. Bark is useful in urinary discharge and piles. The gum Kino is externally applied to leucorrhoea[3]. Combined with other substances of plant origin, some of the metal have fundamental role in the body defense mechanism against certain injurious diseases and few are responsible for certain nasty diseases. Taking into account this fact and broad use of *Pterocarpus marsupium,* the drug has been examined and standardized inorganically from pharmacological point of view.

MATERIALS AND METHODS

Collection and processing of the plant material

Plant material was collected from the hilly regions Radhanagari tahasil of Kolhapur district. Plant was authenticated as *Pterocarpus marsupium* in the herbarium, Department of Botany, Shivaji university Kolhapur Maharashtra, India and voucher specimen was deposited (Herbarium No. SUK-2231). In the winter season the bark was collected in the month of January and summer collection was followed in the month of May. The bark samples were cut into pieces, sun-dried then oven dried at 60^0C. Dried bark samples were ground into powder and stored in an air tight plastic container.

Total nitrogen content

Total nitrogen from the bark was estimated according to the method described by Hawk *et al.*[4]. Plant material, 0.5g oven dried powdered bark was taken in Kjeldahl's flask. To this a pinch of microsalt (200g K_2SO_4 + 5g $CuSO_4$ dehydrated) and 5ml H_2SO_4 (1:1) was added. To avoid bumping few glass beads were added to the flasks and the material was digested on low flame. Faint yellow coloured solution obtained after complete digestion cooled to room temperature and diluted to 100ml with distilled water. The solution was filtered through Whatman No. 1 filter paper and used for estimation of total nitrogen.

2ml plant extract was taken in a set of Nessler's tubes. To each of this tube, one drop of 8% $KHSO_4$ was added and volume was adjusted to 35ml with distilled water. 15ml of freshly prepared Nessler's reagent (Reagent A: 7g KI + 10g HgI_2 in 40ml distilled water, Reagent B: 10 NaOH in 50ml water. 'A' and 'B' were mixed in proportion of 4:5 only at the time of estimation) was added to each test tube. The reaction between sample and the reagent gave orange brown coloured product of NH_4 Hg_2 I_3. The intensity of this colour was measured at 520nm on a double beam UV- spectrophotometer (Shimadtzu UV-190) after 15 minutes. Total nitrogen was calculated with the help of standard curve obtained by using different concentrations of standard ammonium

sulfate solution and employing the similar procedure as described for the analysis of samples.

Preparation of acid digests

Acid digestion method developed by Toth *et al.*[5] was followed for the analysis of inorganic constituents. 0.5g oven dried bark powder was transferred to 100ml capacity beaker and 20ml concentrated HNO_3 was added to it. The beaker was kept covered with watch glass till the primary reactions completed. The beaker was heated gently on hot plate to dissolve solid particles of bark powder. After cooling to room temperature, 60%, 10ml perchloric acid was added to it and mixed thoroughly. The beaker was again heated strongly until a clear 2-3ml colorless solution was obtained. The beaker was cooled and contents in the beaker were diluted to 100ml with distilled water and kept overnight. On the next day, acid digest was filtered through ashless filter paper (Whatman No.44) and filtrate was used for estimation of different inorganic constituents.

Phosphorus content

The phosphorus content was estimated according to the method developed by Sekine *et al.*[6]. Phosphorus reacts with molybdate-vanadate reagent (MV reagent) to give yellow colour complex. The intensity of yellow coloured complex was estimated by using spectrophotometer and by comparing with the colour intensity of the known standards, phosphorus content was estimated. 2ml acid digest was taken in the test tube and equal amount of 2N HNO_3 was added followed by 1ml freshly prepared molybdate-vanadate reagent. Final volume in test tube was adjusted to 10ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes, colour intensity was measured at 420nm using a blank containing no phosphorus. Total phosphorus was calculated with help of standard curve obtained by using different concentrations of standard phosphorus solutions. Other steps are essentially similar as described above. Phosphorus in the plant material was expressed in $g.100g^{-1}$ on dry weight basis.

The levels of Calcium, Magnesium, Iron, Manganese, Copper, Zinc, Chromium, Cadmium, Nickel and Lead were estimated using atomic absorption spectrophotometer (AAS-Model Perkins Elmer 3030). In case needed, appropriate dilutions of plant extract were made with distilled water.

RESULTS AND DISCUSSION

Results of the present investigation are shown in the Fig. 1, Fig. 2 and Fig. 3. Nitrogen is most essential mineral nutrient for plants. It is integral part of the nucleic acids DNA and RNA, proteins, some of the plant growth regulators such as IAA, cytokinins and vitamins. Total nitrogen recorded was higher in the apical stem bark (3.13%) during summer and mature inner bark (1.50%) accumulated lowest total nitrogen during winter. Total nitrogen content in the middle bark was varied from 0.54% during winter to 1.22% during summer. Total nitrogen in the bark of black locust varied from 3.43% to 3.45%[7]. This level of total nitrogen is higher than nitrogen content determined for *Pterocarpus marsupium* in present investigation. Phosphorus, obligatory for plant growth and reproduction, is often referred as "energizer", since it helps to amass and relocate energy during photosynthesis. It is furthermore ingredient of genetic material of all cells DNA and RNA. In the recent survey of phosphorus content among the three bark samples during both seasons, higher intensity was reported in apical stem bark (0.163%) during summer whereas, lowest peak was observed in mature inner bark (0.023%) during winter. Phosphorus contents were moderate in middle bark (0.069-0.084%). Davidson and Le Clerc[8] estimated 0.98%, 0.95%, 0.56% and 0.70% in Lettuce, Spinach, Kale and Broccoli respectively, which is higher than the phosphorus levels in the present examination. In plants, Calcium occurs as free Ca^{+2} as divalent cation. It plays an important role in cell division and cell elongation[9]. Deficiency of Calcium results in osteoporosis[10]. Level of this element was higher in the mature inner bark (1.848%) during winter and lowest value was recorded in apical stem bark (0.60%) during summer. These values are lower than the *Syzygium rotundifolium* (2.6%)[11]. Calcium content in the middle bark was found to be less fluctuated over the seasonal dynamics. Magnesium is strongly electropositive, mobile and abundant divalent element in the plant and average requirement for optimal plant growth varies 0.5%-1.0%13. Magnesium functions as a cofactor for many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes[12]. Level of Magnesium was higher in mature inner bark during summer (0.34%) and lowest level was reported in apical bark (0.21%) during summer while, middle bark contained 0.21-0.23% Mg. These values are higher than the *Calophyllum walkeri* (0.023%)[11].

Copper is essential for the proper functioning of these copper-dependent enzymes, including cytochrome-C oxidase (energy production), superoxide dismutase (antioxidant protection), tyrosinase (pigmentation) and dopamine hydroxylase (catecholamine production)[13]. Copper deficiency increases LDL-cholesterol and decreases HDL-cholesterol, resulting in an increase in cardiovascular disease risk[14]. In current investigation copper peaks from its maximum level 3.2mg /100g in

apical bark during winter to lowest value 0.68mg/100g in mature inner bark during summer. Copper reported in present is higher than *Syzygium rotundifolium* (0.038mg/100g)[11]. Zinc is ubiquitous in plants, microorganisms, and animals. Zinc is present in all body tissues and fluids. Zinc intimately involved in protein, RNA, and DNA synthesis[15]. The apical bark (3.62mg/100g) accumulated maximum amount of zinc during winter, accounting moderate values for middle bark (2.02-2.24mg/100g) and lowest in mature inner bark (1.44mg/100g) during winter. Amount of Zinc estimated in *Justicia adhatoda* (6.585mg/100g)[16] is remarkably higher than reported for *Pterocarpus marsupium* in the present analysis. Manganese (Mn) is distributed in tissues throughout the body and largely located in the mitochondria. Manganese activates enzymes associated with fatty acid metabolism, protein synthesis and involved in neurological function[17]. Manganese is required for normal thyroid function and is involved in the formation of thyroxin[18]. In the present assessment, a high concentration of Mn was scrutinized in the apical stem bark (4.94mg/100g) during summer followed by sensible in middle bark (3.3-4.06mg/100g) and low down in mature inner bark (2.0mg/100g) during winter. Manganese quantity confirmed in the stem tissue of *Vaccinium myrtillus* (26.52mg/100g and *Convalaria maialis* (17.63mg/100g)[19] is much greater than resolved for bark of *Pterocarpus marsupium* in the present issue. Iron, is one of the most important micronutrient required for various metabolic activities in plant and animal the cell. Longstanding iron deficiency reduces physical working capacity in human which improved after iron administration[20]. Iron estimated in the apical bark (44.34mg/100g) during winter was much higher when compared with mature inner bark (11. 38-17.38mg/100g). The iron composition in the middle bark (32.14-39.36mg/100g) hits in between apical and mature inner bark. These values are much lower than *Alternanthera pungens* (73.95mg/100g) and *Brassica campestris* (188.97mg/100g)[16].

Chromium (Cr) is not naturally present in the earth crust. Trivalent chromium (Cr^{3+}) is most found in living organisms. Chromium participates in gene expression by binding to chromatin, causing an increase in RNA synthesis by induction of protein bound in the nucleus and nuclear chromatin activation[21]. Chronic exposure to Cr causes nose septum perforation and small cell cancer of the lung[22]. In the present study Chromium concentration among the three bark samples was found maximum in mature inner bark (3.94mg/100g) during winter and lowest in apical bark (2.08mg/100g) during summer. Moderate Cr concentration was noticed for middle bark (2.44-3.74mg/100g). These values are very high than the Cr content in spinach (0.660mg/100g)[23] and clover shoot (0.265mg/100g)[23]. Nickel is widely distributed in the environment, and can be found in air, water, soil and the level of nickel in ambient air is small (about 6-20 ng·m-3)[24]. In biological systems, Ni+2 form of Nickel is the most common[25]. High concentration of nickel causes lung and nasal cancer [26], dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract[27]. While scrutinizing the bark of Pterocarpus marsupium highest concentration of Nickel was reported in the mature inner bark (1.26mg/100g) during winter. Nickel portion in the Middle bark fluctuate between (0.66-0.86mg/100g). Amount of Ni was reduced to its lowest value in apical stem bark (0.34mg/100g) during summer when compared with middle and mature inner bark. Jabeen et *al*.[16] evaluated lower concentration of Ni in *Withania somnifera* (0.566mg/100g) and *Justicia adhatoda* (0.40mg/100g) when compared with Nickel values estimated in the present study. Cadmium is regularly found in ores together with zinc, copper and lead. Foods contaminated with cadmium causes vomiting and acute gastrointestinal effects[28], While, chronic exposure cause to kidney and spontaneous abortions. According to WHO, 0.3mg/Kg of lead is set as the threshold limit for safety. Exceeding this limit, it results in lead poisoning, blindness, deafness, hypertension, impairment of kidney function and neurological disorder[29]. Either of the bark samples of *Pterocarpus marsupium* under present investigation showed absence of cadmium and lead, the two hazardous entities.

CONCLUSION

The bark samples of *Pterocarpus marsupium* contain minerals in appreciable amount. The inorganic data revealed in this act will provide valuable base for drug prescription and at time of drug designing. By knowing the concentration of respective element, drug in its crud form can be applied in substrate deficient conditions. Inorganic report evaluated here will cooperate while ensuring the quality and verifying adulteration in the natural drug and in the formation of various Ayurvedic medicines.

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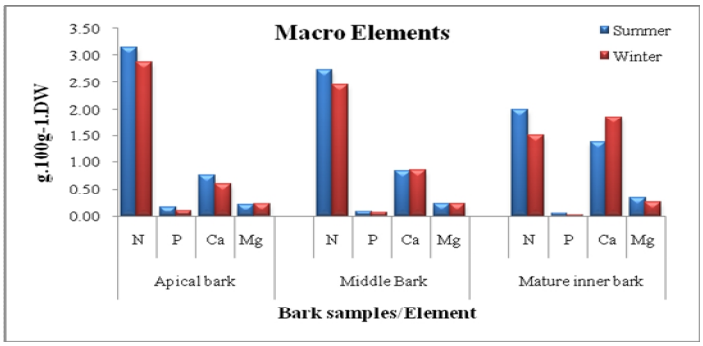

Fig.1. Quantitative estimation of macro elements (N, P, Ca and Mg) from *Pterocarpus marsupium* bark

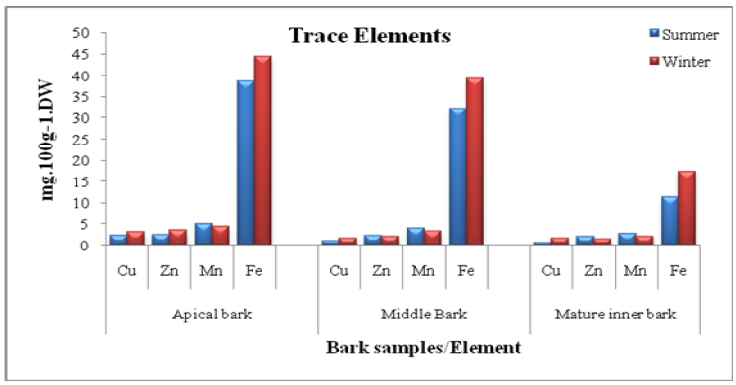

Fig.2. Quantitative estimation of micro elements (Cu, Zn, Mn and Fe) from *Pterocarpus marsupium* bark

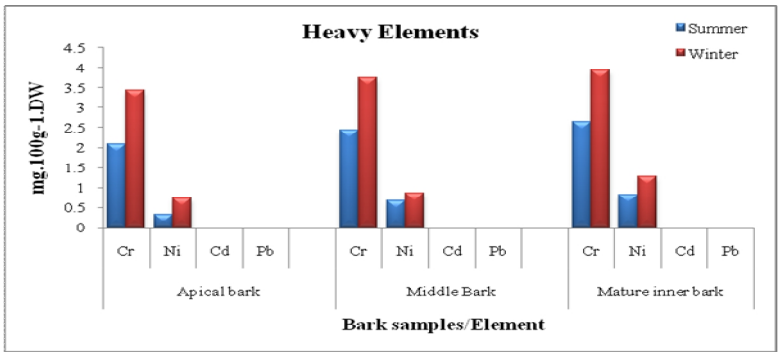

Fig.3. Quantitative estimation of heavy elements (Cr, Ni, Cd and Pb) from *Pterocarpus marsupium* bark

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