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Original Article

Nutritional Profiling and Anti-Oxidant Activity of *Teramnus Labialis (L.F) Spreng* Seeds

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ABSTRACT

Seeds of *Teramnus labialis* has some nutritional values, according to the study *Teramnus* plant are rich source of bioactive chemicals that have potential to prevent and treat illness. These seeds were analyzed for protein, starch, reducing sugar, total sugar, antioxidant activity and vitamins content. Protein value was 26.62 mg/g, 0.98 mg/g total sugar, 0.373 mg/g starch and 0.1 mg/g reducing sugar is estimated. The antioxidant assay has been performed for estimation of reducing power of fruit DPPH, ABTS, CUPRAC and PMA. Vitamins A was 20.82g/100g, vitamins C 1.272mg/100g and vitamins E3.4974µg/g obtained.

Keywords: Antioxidant assay, biochemical assay, *Teramnus labialis*, vitamins

INTRODUCTION

Teramnus labialis is a rich source of compounds that may be used to cure and prevent many diseases, many study show diterpenoids, flavonoids, and iridoids are examples of these compounds, which have a variety of properties including antibacterial, anti-inflammatory, and antioxidant properties. Thus, they could help with anything from cancer treatment to infection prevention to liver protection. Despite the need for more research, it is clear that *Teramnus*plants have the potential to play a huge role in medicine (Vadivel and Janardhanan, 2005.). Legumes are consumed all over the world and provide a substantial amount of nutrients for human diets (Wang *et al*., 2003, Grusak*et al*., 2005). They provide a variety of vitamins, a huge number of essential minerals for human health, protein, and fats or carbohydrates for energy. Several legume species have been employed for large-scale production in a range of climatic zones, even though many wild species are also gathered and consumed on a smaller scale by rural or tribal communities (Rodrigues and Torne, 1991, Janardhanan *et al.*, 1995, Seena *et al*., 2005). Many people are interested in developing these wild legumes into cultivars, especially as efforts to increase the mineral content of human seed meals have increased in recent years (Bouis *et al*., 2003.). Among these wild legumes, *Teramnus labialis* Spreng has received very little study as a grain crop. Asia, Africa, and the Americas are home to tropical regions where this legume is indigenous (Verdcourt *et al*., 1970). It has been reported that some South Indian tribal communities eat it as a seed (Viswanathan *et al*., 1999)

1.1 Vernacular Names:

Sanskrit- Mashaparni
English- Vogel-blue wiss
Marathi- Ran Udid
Hindi- Jungliudad
Gujarati-Banudad,adad

1.2 Taxonomic position according to Bentham and Hooker, 1883

Kingdom – Plantae
Phylum- Angiosperms
Class – Magnoliopsida
Order – Fabales
Family – Fabaceae
Genus – Teramnus
Species – labialis

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



MATERIAL AND METHODS:

2.1 Sample Collection

The plants were collected in January 2025 from the Gujarat University Campus, Ahmedabad district, Gujarat, India. The plant herbarium is authenticated by the Department of Botany, Gujarat University. The voucher specimen was deposited at the herbarium of the Botany department, at Gujarat University, Ahmedabad.

2.2 Extract preparation

After being cleaned with distilled water, the fruits were dried in an oven at 50 ± 1°C. A mortar and pestle were then used to grind the dried fruits into a powder. Methanol and acetone were used as solvents in the Soxhlet extraction procedure to create the extracts. 10mL of solvent were used to extract fifty milligrams of powdered material. After filtering the extract through Whatman No. 1 filter paper, it was left to dry in Petri dishes for a full day AOAC (2005).

2.3 Biochemical Estimation of the Following Metabolites:

2.3.1Protein: Standard protocol of total protein by Bradford, 1976, was followed:
Grind 1 gm plant material in 10 ml of 0.1M phosphate buffer (pH 7.2) using mortar and pestle. Centrifuge the extract at 10,000 rpm for 15 minutes at 4°C. Use the supernatant as extract for estimation of total soluble proteins. Prepare various concentrations ofthe standard protein solution from the stock solution (i.e. 0.2, 0.4, 0.6, 0.8, and 1.0 ml) into series of test tubes and make up the volume to 1 ml by adding distilled water and pipette out 0.2 ml of the sample in two other test tubes and make up the volume to 1 ml by adding distilled water. A tube with 1 ml of distilled water serves as blank. Add 5.0 ml of Bradford reagent to each tube and mix by vortex or inversion. Wait for 10-30 minutes and take a reading of the standard and sample at 595 nm. Plot the absorbance of the standard versus its concentrations. Plot the graph of optical density versus concentration. From the graph, find the amount of protein in the unknown sample. Determine the concentration of protein using BSA as standard. The result was expressed as (mg/ml) plant material

2.3.2. Starch: Standard techniques for estimating starch were provided by Hodge and Hofreiter, 1962. To eliminate sugars, homogenize 0.1 to 0.5 grams of the sample in hot 80% ethanol. Keep the residue after centrifuging it .Repeatedly wash the residue with hot 80% ethanol until the anthrone reagent no longer imparts color. The residue should be thoroughly dried over a water bath then add 5.0 ml of water and 6.5 ml of 52% perchloric acid to the residue. Keep the extract at 0°C for 20 minutes. save the supernatant after centrifuging. Using additional perchloric acid, repeat the extraction process and make up to 100 ml by centrifuging and collecting the supernatant. Using a pipette, remove 0.1 or 0.2 ml of the supernatant and add water to reach 1 ml. Assemble the standards by filling each test tube with water and adding 0.2, 0.4, 0.6, 0.8, and 1.0 ml .Fill each tube with 4 ml of the anthrone reagent. In a boiling water bath, heat for eight minutes. Quickly cool and measure the green to dark green color intensity at 630 nm.

2.3.3. Reducing Sugar: Somogyi, M. (1952) established the standard procedure for completely reducing sugar. Weigh 100 mg of the sample and extract the sugars with the hot 80% ethanol twice (5 ml each time), Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10 ml water and dissolve the sugars after it pipette out aliquot of 0.1 or 0.2 ml to separate test tubes. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution into a series of test tubes Make up the volume in both standard and sample tubes to 2.0 ml with distilled water. Pipette out 2.0 ml of distilled water in a separate test tube to set a blank. Add 1 ml of alkaline copper tartrate reagent to each tube. Place the tubes in boiling water for 10 minutes after cooling the tubes add 1 ml of arsenomolybdate reagent to all the tubes. Make up the volume in each tube to 10 mL

with water. Read the absorbance of the blue color at 620nm after 10 min
Calculation:Absorbance corresponding to 0.1 ml of test = 'X' mg of Glucose 10 ml contains = ('X'/0.1) × 10 mg of glucose = % of reducing sugars

2.3.4. Total sugar:

Standard method of Nelson, 1944 was used for total sugar estimation. 100 mg fresh plant material is crush with 10 ml 80% boiling ethanol and centrifuge for 10 mins at 5000 rpm.Collect supernatant 0.2 ml in test tube and add 1ml 1N sulphuric acid and incubate for 30 min at 49°**C**. After it add 1-2 drops methyl red indicator and 1N NaOH colour changes from pink to yellow.Add 1 ml Nelson reagent and incubate for 20 mins in waterbath.1 ml arsenomolybdateis added after it make final volume upto 20 ml with distilled water.Take absorbance at 620 nm.

2.4 Estimation of Vitamins

2.4.1 Vitamins A

Vitamin A was determined by method of Onyesife*et al*., (2014). Grind 5g of sample by adding 1ml of saponification mixture i.e 2N KOH in 90% alcohol.Heat tubes for 20 min at 60temperatures afterwards cool it down and add 20 ml water to it. Extract vit A with 10 ml petroleum ether in separating funnel.Pull out the extract and add sodium sulphate. Evaporate 5ml aliquot of ether-extract to dryness then dissolve 1 ml chloroform in residue.pipette out standard to a series of 1.5-7.5µg and make up the volume to 1 ml with chloroform then add 2 ml TCA solution. Record absorbance at 620nm.Construct standard graph plotting the A620in y-axis and vit A concentration in x -axis. Calculate the amount of vitamin A/g tissue of samples. Calculation: y=mx-c

2.4.2 Vitamin C

Vitamin C estimation was done by volumetric method, described by Harris and Ray (1933). Pipette out 5 ml of working standard solution into 100ml flask in that add 10 ml 4% oxalic acid and titrate against the dye (V1 ml).End point is appearance of pink colour (persist for few mins only), the amount of dye consumed is equivalent to the amount of ascorbic acid.Next extract the sample 0.5-5g in 4% oxalic acid and makes up a known volume i.e 100 ml and centrifuge it.Pipetteout 5 m of this supernatant, add 10 ml of 4% oxalic acid and titrate it against due (V2 ml)
Calculation:
Amount of ascorbic acid mg/100g sample = 0.5/V1 ml* V2/5ml * 100 ml/wt. of sample*100

2.4.3 Vitamin E

Vitamin E estimation was done by the method described by Rosenberg (1992). Grind 0.5g sample and pour it into stopper tube by slowly adding 0.1N sulphuric acid without shaking.Allow it to stand overnight then filter it on next morning by Whatman no. 1 filter paper.use aliquots for practical.Pipette. it out 1.5ml into three centrifuge tubes (test,standard and blank).Afterwards add 1.5 mlxylene mix it thoroughly and centrifuge,transfer 1ml of xylene layer into another tube.Add 1 ml of 2,2' dipyridyl reagent to each tube and mix again.Pippete out 1.5ml of mixture into cuvette,read the extinction of test and standard against blank at 460nm.Then add 0.33ml ferric chloride to each tube, after exactly 15 min read the test and standard against blank at 520 nm
Calculation:
amount of tocopherol in µg/g of tissue= reading of test at 520 nm -reading of test at 460 nm/reading of standard at 520nm*0.29*15*total vol of homogenate/vol used *weight of tissues

2.5 Antioxidant assay

2.5.1 DPPH radical scavenging assay:

The conventional approach of Brand-Williams *et al*., 1995 was used, with minimal modifications, to assess the DPPH (1,1 Diphenyl 2 Picrylhydrazyl) radical scavenging activity. After freshly preparing the DPPH stock solution (4 mg in 100 ml methanol), it was left in the dark for 30 minutes. Standard ascorbic acid (0-100 µl from stock 1 mg/ml) with plant extract were transferred to a test tube, where methanol was used to create the final amount of 1 ml. After actively shaking 3 milliliters of DPPH with the test tube solution, it was left in the dark for 30 minutes. The mixture's absorbance was measured at 517 nm using a Shimadzu UV-1800 (Shimadzu Corporation, Kyoto, Japan) in comparison to a blank of DPPH-free pure methanol. The results were expressed as percentage inhibition of DPPH according to the following formula:
I% = (Acontrol-Asample)/ (Acontrol)×100

Where $A_{control}$ = absorbance of the DPPH solution without extract; A_{sample} = absorbance of the DPPH solution with plant extract.

2.5.2 ABTS radical scavenging assay

[2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity was determined by Patel and Ghane in 2022. In a test tube, standard Trolox (0-200 µl from stock 4 mg/ml) and plant extract (0-200 µg/ml) were combined, and with the aid of methanol, the final volume was 1 ml. After adding 3 ml of ABTS reagent, the mixture was incubated for 30 minutes. In comparison to the blank (methanol without ABTS reagent), the absorbance was measured at 730 nm. The results were expressed as a percentage of $ABTS^+$ scavenging according to the following formula: I% = (Acontrol-Asample)/ (Acontrol)×100

Where Acontrol = absorbance of the ABTS solution without extract; Asample = absorbance of the ABTS solution with plant extract.

2.5.3 CUPRAC assay

Using the Sethi *et al*., 2020 technique, the Cupric Reducing Antioxidant Capacity assay was carried out. 0.2 ml of sample extract or standard Trolox (0.125-1 ml) was used for the test. Methanol was added to get the final volume up to 1 ml, and 3 ml of CUPRAC reagent (copper chloride, neocuproine, and ammonium acetate buffer (pH 7) solution in a 1:1:1 ratio) was added to create a total reaction mixture of 4 ml. Determine the absorbance at 450 nm following a half-hour incubation period at room temperature. The results were given as milligrams of Trolox equivalents (TE) per gram of extract.

2.5.4 Phosphomolybdenum assay (PMA)

The Prieto *et al*., 1999 method was used to perform the phosphomolybdenum assay. The approach was slightly altered in this case. In this experiment, phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was combined with 0.2 ml of plant extract (1 mg/ml stock) or standard ascorbic acid (0.2-1 ml). After that, the reaction mixture spent sixty minutes in the water bath. At 695 nm, absorbance was measured after cooling to ambient temperature. Ascorbic acid equivalents (AAE) in milligrams per gram of extract were used to express the antioxidant capacity.

2.6 Statistical Analysis:

Experimental results were analyzed as the mean ± S.D. of three independent replicates for Biochemical, vitamins and antioxidant assay. Statistical analysis was carried out using Microsoft® Office Excel (Microsoft® U.S.A).

RESULTS AND DISCUSSION:

3.1 Biochemical assay:

Primary metabolism is important for growth, development and reproduction of cells. The primary metabolites participate in the primary response by the regulation of carbohydrates, protein and lipids to infection by pathogens (Rojas *et al*., 2014)

Table 1: Biochemical composition of seed of *Teramnus labialis*

Sr. No.	Plant Sample	Protein	Total Sugar	Reducing Sugar	Starch
1	Fresh Seeds	26.62 ± 0.13 mg/g	0.1± 0.04mg/g	0.373± 0.1mg/g	0.98± 0.07mg/g

3.2 Vitamins

3.2.1 Vitamin A: Estimation of vitamins A by following standard method, Onyesife *et al*., (2014).

Y=mx+C, Y=0.0984x-0.0009, R2=0.9999

2.048+0.0009/0.0984=20.82mg/100ml

3.2.2 Vitamin C: Estimation of vitamins C by following standard method,described by Harris and Ray (1933).

Calculation: Amount of ascorbic acid mg/100g sample = 0.5/V1 ml* V2/5ml * 100 ml/wt. of sample*100

Here, V1= 11.5

V2 = 7.4

0.5/11.5*7.4/5*100/0.5*100

=1.272 mg/100 ml

3.2.2 Vitamin E: Estimation of vitamins E by following standard method,Rosenberg (1992)

amount of tocopherol in µg/g of tissue= reading of test at 520 nm -reading of test at 460 nm/reading of standard at 520nm*0.29*15*total vol of homogenate/vol used *weight of tissues
=0.938-0.402/0.500*0.29*15*2/1.5*0.5
=4.6632*0.75
=3.4974µg/g
Amount of vitamin E in dry seeds is 20.82g/100g, amount of vitamin C is 1.272mg/100g and amount of vitamin E is 3.4974 µg/g is estimated in *Teramnus labialis* dry seeds

The idea that plant constituents with antioxidant activity can exert protective effects against oxidative stress in biological systems has been strongly supported by epidemiological and in vitro studies on medicinal plants and vegetables (Cao *et al*., 1996, Block and pattersson 1996). Oxidative stress-induced ROS and free radicals are thought to be a major cause of physiological disorders like Parkinson's, arthritis, atherosclerosis, coronary heart diseases, emphysema, gastric ulcers, diabetes mellitus, cirrhosis, aging, and cancer (Singh *et al*.,2009).

3.3 Anti-oxidant activities

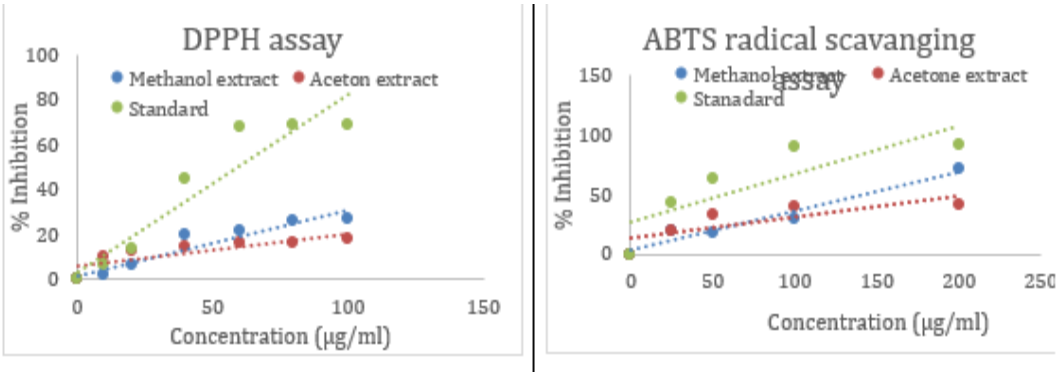


Fig.1(A) and (B) % Inhibition of plant sample and standard

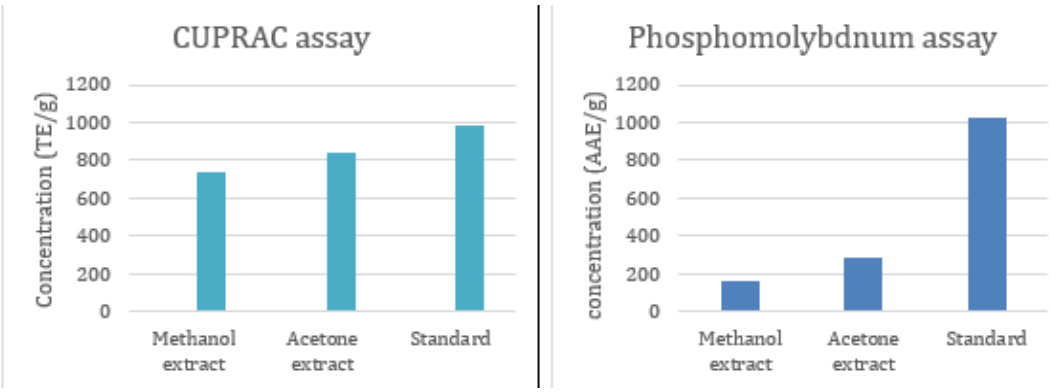


Fig.3 reducing power of antioxidant agent in plant sample and standard

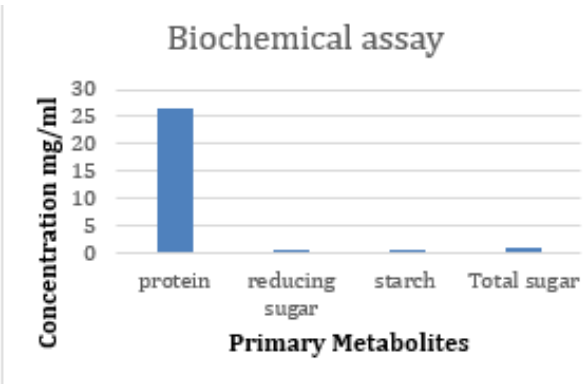


Fig. 1 Biochemical assay of Teramnus labialis

CONCLUSION:

From this research we estimated that seeds of *Teramnus labialis* contain many primary metabolites like carbohydrates. protein, starch, sugar, retinol, tocopherol and ascorbic acid. As primary metabolites are primary precursors for the production of secondary metabolites their analysis is necessary.Hence,*Teramnus*contains high amount of protein it cansbe a good source of protein for cattle's as well as human consumption.

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