PRELIMINARILY replace with preliminary PHYTOCHEMICAL SCREENING, ANTI INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF MACROTYLOMA UNIFLORUM (HORSE GRAM) LEAF ETHANOLIC EXTRACT ON SELECTED ORAL PATHOGENS

Running title: Preliminary phytochemical activity of Macrotyloma Uniflorum leaf ethanolic extract.

ABSTRACT:

AIM: To know the Preliminary phytochemical screening, Anti Inflammatory activity and antimicrobial activity Macrotyloma uniflorum (Horse gram) leaf ethanolic extract on selected oral pathogens.

INTRODUCTION: Macrotyloma uniflorum also known as horse gram is a plant grown for feeding horses, rarely by humans and also in Ayurvedic cuisine. This plant is a legume from tropical southern Asia which belongs to the family of Fabaceae and the kingdom of Plantae.

MATERIALS AND METHODS: Macrotyloma uniflorum ethanolic plant extract was made and statistical analysis was done using various methods in order to know the anti inflammatory and antioxidant properties by preliminary phytochemical screening. BRIEFLY RE-WRITE METHOD

RESULTS AND DISCUSSION:The polyphenolic compounds flavonoids and tannins appear to be the most promising in Macrotyloma uniflorum ethanolic plant extract.

CONCLUSION: The study demonstrated in vitro antioxidants and anti- inflammatory activities of Macrotyloma uniflorum leaf extract through scavenging, chelating and reducing activity.

KEYWORDS: Ethnolic extract, Macrotyloma uniflorum, preliminary phytochemical screening, Antioxidant, Anti Inflammatory activity, Innovative technology, Novel method

INTRODUCTION:

Macrotyloma uniflorum also known as horse gram is a plant grown for feeding horses, rarely by humans and also in Ayurvedic cuisine(1). This plant is a legume from tropical southern Asia which belongs to the family of Fabaceae and the kingdom of Plantae(2). There are so many benefits of eating horse gram like it has good benefits diarrhoea, conjunctivitis, weight loss, Menstrual disturbances, constipation, skin rashes etc but there are also side effects like allergic reactions in some people and also gas and bloating as it contains phytic acid but this can be reduced by cooking, soaking it in water and sprouting(3).

Preliminary phytochemicals screening is a very useful step in order to know the bio active principles present in medicinal plants and this might help us in finding new drugs and developing further in order to make it useful for living beings to survive and keep them healthy(4). Phytochemical screening is very important because chemical substances can be therapeutically active or it also can be inactive, this phytochemical screening will help us in finding bio active profile of plants and its therapeutic importance in the field of medicine. There are various phytoconstituents like alkaloids, flavonoids, steroid, terpenoid, tannins, saponins, cardiac glycosides and anthraquinones(5). The presence of these phytoconstituents can be done using various tests like Dragemdorf Mayer, Wagner test, Alkaline reagent and Shinoda test, Liebermann Burchard test and Salkowski test, FeCl2 test, Foam test, and Killer-Killiani test(6,7). Positive results from these tests indicate the presence of various phytoconstituents given above(8).

Antimicrobial activity is a process of inhibiting the growth of microorganisms that is causing various diseases(9). Antimicrobials can be either antibacterial or antifungal or antiviral. They act by different mechanisms in order to prevent us from various microbial infections. Plants are naturally enriched with secondary metabolites like tannins, terpenoids, alkaloids, and flavonoids which usually proved to have antimicrobial properties(10,11). Antimicrobial activity can be performed using various techniques such as Agar well diffusion methods, Agar plug diffusion method, Cross streak method, Poisoned food method etc(12). There are various other plants which have natural antimicrobial action that are very useful in preserving food from getting spoiled from microorganisms and also different types of essential oil have been extracted from

the same(13). Anti inflammatory activity will help in reducing any inflammation or swelling.

REWRITE THE INTRODUCTION BY NARROWING ON THE PLANTS SPECIFICALLY

There are several researchers telling us about the antibacterial, antiurolithiatic activities of this macrotyloma uniflorum plant(13). Let us know whether this plant has Antiinflammatory and antimicrobial activity using preliminary phytochemical screening of leaf ethanolic extract on selected oral pathogens.Our team has extensive knowledge and research experience that has translate into high quality publications (14),(15),(16),(17),(18),(19),(20), (21),(22), (23), (24), (25), (26),(27),(28),(29),(30),(31),(32),(33) REPHRASE PLEASE. THERE IS ALSO NO NEED FOR WRITING THIS NUMEROUS NUMERICAL REFERENCES

The main aim of our study REWRITE AS THIRD PERSON SINGULAR is to know the preliminary phytochemical screening, Anti Inflammatory and antimicrobial activity of macrotyloma uniflorum leaf ethanolic extract.

MATERIALS AND METHODS:

1) REMOVE AND CITE AT THE END OF THE STATEMENT The plant Macrotyloma Uniflorum was taken and was ground into powder. One gram of the ground powder was properly measured and taken. This USE 3RD PERSON SINGULAR was then added to 100ml of water and mixed well. This mixture was then boiled for 5-10 minutes. After boiling, using a filter paper, filtering funnel and measuring cylinder, the solution was filtered and the filtrate was obtained and then the filtrate was used to know the anti-inflammatory and antimicrobial properties of this plant. Later in vitro Anti Inflammatory and Antimicrobial activity was done using various criteria.

2)In Vitro anti-inflammatory activity:

Protease inhibition assay:

The method of Oyedepo and Femur Was used to assess trypsin inhibition (1965). METHOD IS OBSOLETE, USE RECENT METHODS

100 µL of bovine serum albumin was added to 100 µl of plant extracts (0.1 to 0.5mg/ml) with increase in concentrations (100-500µg/ml).

The prepared extract was incubated for 5 minutes under room temperature. Reaction was inhibited by the addition of 250 µl of trypsin followed by centrifugation.

The supernatant was filtered, and the absorption spectra at 210 nm was determined. As a positive regulation, acetyl salicylic acid was used.

The experiment was repeated three times and the percent inhibition of protease inhibition measured. Aspirin was used as a normal anti-inflammatory drug in this research.

Calculation:

% Inhibition=100−((A1 −A2)/A0)*100) USE EQUATION EDITOR

3) CITE REFERENCE AT THE END OF THE METHOD In Vitro antimicrobial activity

DPPH radical assay The DPPH free radical scavenging assay was performed by LiyanaPathirana and Shahidi method [Kikuzaki and Nakatan, 1993]. 200 µL of 0.1 mM DPPH prepared in methanol was added to 100 µL of the plant extract with increase in concentration (100-500µg/ml).

The mixture was again incubated under the room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. BHT was taken as a positive control. The experiment was repeated three times, with the percentage inhibition of DPPH radical scavenging behaviour measured.

% Inhibition=((A0 -A1)/A0)*100 USE EQUATION EDITOR

The absorbance of the control is A0, and the absorbance of the sample is A1.

4)Statistical analysis:

The data were analyzed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

RESULTS:

Preliminary phytochemical results indicated the presence of steroids, Flavonoids, phenols, Alkaloids and carbohydrates (Table: 1). As the concentration increased, the percentage of inhibition also increased. Accordingly, Macrotyloma uniflorum might contain a sizable amount of reductants which may react with the free radicals to stabilize and terminate from free radical chain reaction. Phenolic and flavonoid compounds can play a significant role in antioxidant activity(REFERENCES). Macrotyloma uniflorum has a significant amount of total phenolics and flavonoids. Methanolic extract of Macrotyloma uniflorum has shown higher in vitro

antioxidant activity. The polyphenolic compounds flavonoids and tannins appear to be the most promising (Fig: 1). The extracts displayed greater inhibition of protein denaturation. The results clearly indicate that this plant has potential as an anti- inflammatory agent, thus making this study even more significant. MPE exhibited anti- inflammatory activity via inhibition of the production of NO (57.8%), PGE2 (97.1%) and IL-6 (93.2%). MPE inhibited the development of IL-1 (60.9%), TNF- (37.9%), and IL-6 (40.9%), as well as the synthesis of MMP-2, MMP-9, and COX-2 (fig: 2).

Test	**Ethanolic extract**
Steroids	+
Triterpenoids	-
Flavonoids	+
Phenols	+
Tannins	–
Alkaloids	+
Saponins	-
Acid	-
Carbohydrates	+
Glycosides	-
Proteins	-

Table:1: The above tabulation represents Preliminary phytochemical screening of Macrotyloma Uniflorum leaf ethanolic extract. '+' sign is present and '-' is absent. From the above tabulation, we can conclude that Macrotyloma Uniflorum had various steroids, Flavonoids, phenols, Alkaloids and carbohydrates. MOVE IT ABOVE THE TABLE

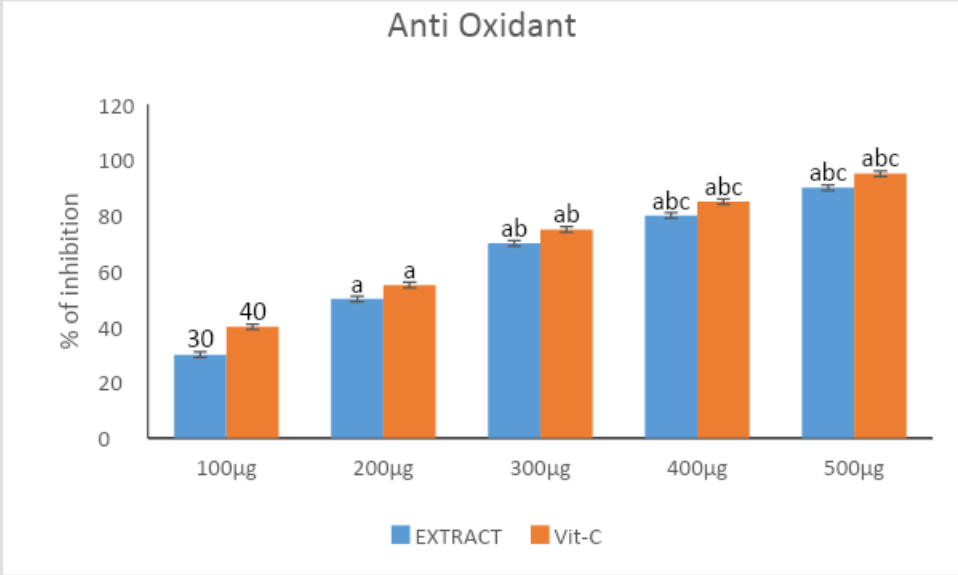

Figure: 1: Anti-antioxidant activity of *Macrotyloma uniflorum*. Each bar represents mean ± SD of 6 observations. Significance at the levels of $p < 0.05$.a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.

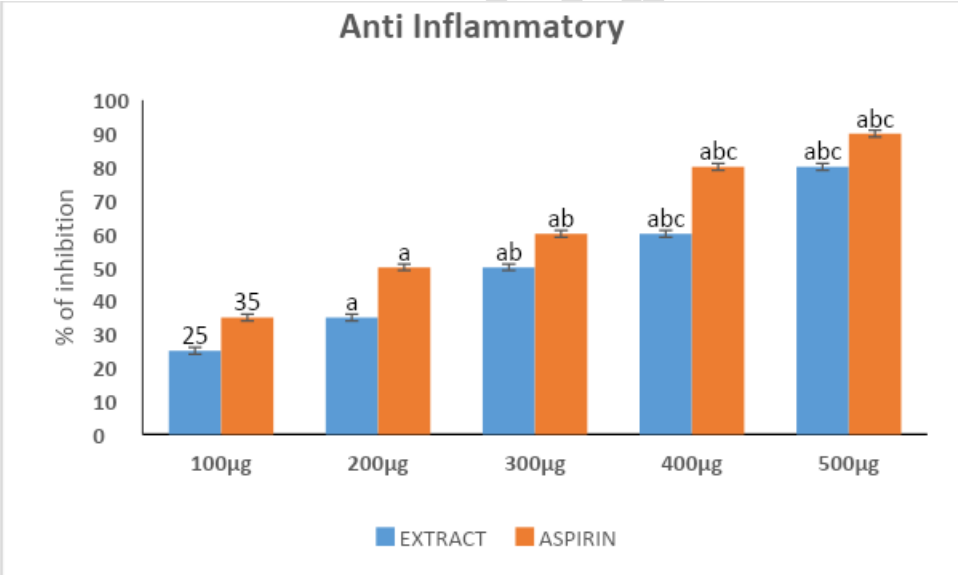

Figure: 2: Anti-inflammatory activity of *Macrotyloma uniflorum*. Each bar represents mean ± SD of 6 observations. Significance at the levels of $p < 0.05$.a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.c-compared with 400µg.

DISCUSSION:

Secondary plant metabolites have been extensively studied as a source of pharmaceutical compounds in recent years(REFERENCES). Natural compounds are widely acknowledged to play an important role in health care(34). Previously done studies on photochemical activity of Macrotyloma Uniflorum extract with methanol confirmed the presence of carbohydrate, protein, amino acid, terpenoids, saponins, flavonoids, alkaloids, steroids, glycosides and phenols. Biological properties of phenolic compounds include antiapoptosis, antiaging, anticarcinogen, anti inflammation, and inhibition of angiogenesis and cell proliferation, according to Singh et al.21. Plants use phytosterol as a growth hormone(35). Because of the presence of these phytochemicals, the plant has medicinal properties. Alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids, terpenoids, proteins, and carbohydrates were identified in various solvent extracts of M. uniflorum seeds by Manikandan et al. through phytochemical screening(36,37). The ethanolic extract, followed by methanol, aqueous, chloroform, and hexane extracts, was found to be the most effective of the various solvents. There is another study stating that fixed oil from Macrotyloma uniflorum may have analgesic and anti-inflammatory properties that are linked to a peripheral mechanism of action(38,39). Inflammation is a complicated process that is frequently associated with pain. There is an increase in vascular permeability, mononuclear cell migration, granulocytes, and granulomatous tissue in this condition(40).

CONCLUSION:

The study demonstrated in vitro antioxidants and anti- inflammatory activities of Macrotyloma uniflorum leaf extract through scavenging, chelating and reducing activity indicated the performed test shows good antioxidant and anti-inflammatory activity. Further invivo studies and clinical trials may be required for drug development in treatment of inflammatory disorders.

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