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EXPLORING THE COMBINED ANTI-INFLAMMATORY SYNERGISTIC POTENTIALS OF *PTEROCARPUS MARSUPIUM* STANDARDIZED BARK EXTRACT AND *CRATAEVA NURVALA* STANDARDIZED BARK EXTRACT

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ABSTRACT

After searching PubMed, Google Scholar, Scopus, and other imperative databases, it was discovered that no combined anti-inflammatory perspectives of *Pterocarpus marsupium* bark extract and *Crataeva nurvala* bark extract have been published in an *in vitro* process (human red blood cells, HRBC). To investigate synergistic behavior, the *P. marsupium* standardized bark extract (PMSBE) and *C. nurvala* standardized bark extract (CNSBE) were screened together. PMSBE supported 42.88% membrane stabilization/protection in HRBCs at a concentration of 250 µg/mL, while CNSBE provided 38.26% membrane stabilization/protection in HRBCs. The anti-inflammatory activity was improved to 59.52% when the extracts PMSBE and CNSBE were measured together (at a concentration of 250 µg/mL each). When all extracts were used at the same time, the anti-inflammatory value increased from 16% to 21%. Flavonoids, phenols, and alkaloids, which are present in plant products, play a crucial role in mediating anti-inflammatory activity by inhibiting multiple inflammatory enzymes. The extract's strong phenolic and flavonoid content worked together to balance the HRBC membrane in a synergistic way. This study will pave the way for further studies into the discovery of novel herbal formulations for the treatment of acute and chronic inflammation.

KEYWORDS: *Pterocarpus marsupium*, *Crataeva nurvala*, Bark, Anti-inflammatory, HRBC Method, Extract.

INTRODUCTION

Inflammation is a reaction in which white blood cells and a variety of important chemical mediators are activated in order to protect us against pathogenic organisms such as bacteria. Although the function is essential for survival, it causes excruciating pain and suffering when it is aggravated.[1] Inflammatory conditions, such as inflammation, lead patients to feel even worse than they do and attack the body's own tissue. As a consequence, anti-inflammatory medications are seldom required to alleviate inflammation and the resulting reactions.[2] Several biochemical processes in the inflammatory phase are catalyzed by enzymes such as cyclooxygenase-1/2 (COX-1/2), lipoxygenase (LOX), prostaglandin synthetase (PGS), prostaglandin dehydrogenase (PGDH), and others, resulting in the formation of leukotrienes, which are important in acute inflammation.[3] A number of anti-inflammatory drugs, both steroidal and non-steroidal, with properties of inhibiting these multiple mediators have attracted pharmacotherapeutic interest, however they have not been widely used for a variety of factors, including poor pharmacokinetics, unintentional side effects, and so on.[4] In order to create an effective prescription, attempts are made on a routine basis to address these anti-inflammatory-related issues using a number of realistic approaches.[5]

Since ancient times, *Pterocarpus marsupium* has been commonly used as a traditional ethnopharmacological variable. The bark and resin decoction is used as an astringent for acute diarrhea, dysentery, gland tumors, urethral discharges, scalp ringworm, recurrent ulcers, and as an abortifacient. Elephantiasis, leucoderma, diarrhoea, rectalgia, cough, burns, fever, stomachache, diabetes, jaundice, antiulcer, and gray hair benefit from the heart wood, which is astringent, bitter, acrid, anti-inflammatory, anthelmintic, and anodyne.[6]

Crataeva nurvala's medicinally active sections include the bark, leaves, base, and vine. The bark is hot, bitter at first, and then sweet sharp taste; easy to digest; stomachic, laxative, anti-lithic, vesicant, anthelmintic, detergent, bechic, expectorants; removes Vata, well in strangury, biliousness. The bark stimulates appetite, reduces bile and phlegm secretions, and heals urinary organ diseases. It also has antipyretic, sedative,

alterative, and tonic properties. In certain cases of urinary symptoms and diarrhea, as well as some minor types of skin disease, the bark is beneficial. The leaves are stomachic, tonic, and rubefacient on the outside, as well as internally febrifuge and tonic. The leaf and bark are lithontriptic and laxative, and the root is often alterative. Root and bark stimulate hunger and bile secretion. The flowers are cholagogue and astringent. The fruit is sweet and sticky, and it acts as a laxative, removing "Vata", "Pitta", and "Kapha".[7]

After searching PubMed, Google Scholar, Scopus, and other prescription databases, it was discovered that no combined anti-inflammatory perspectives of *P. marsupium* bark extract and *C. nurvala* bark extract have been published in an *in vitro* process (human red blood cells, HRBC). To investigate synergistic behavior, the *P. marsupium* standardized bark extract (PMSBE) and *C. nurvala* standardized bark extract (CNSBE) were screened together.

MATERIALS AND METHODS

Chemicals

The standard drug diclofenac sodium was available from the Generic Medicine Store in Bilaspur, Chhattisgarh. The reagents, consumables, and chemicals for this research were obtained from HiMedia® India Pvt. Ltd., Mumbai, through a local distributor. A double-distilled water apparatus (Borosil®, India) was used in the experiment.

Instruments

For spectroscopic analysis, a double-beam Shimadzu® Ultraviolet-Visible Spectrophotometer (Model UV-1800, Japan) was used, which was connected to a computer with a spectral bandwidth of 1 nm and wavelength resolution of 0.3 nm, as well as a pair of 10 mm path period aligned quartz cells. The chemicals were measured using an Accro Tech® electronic balance (Model AT-266-1, India).

Plant materials

Green Heavens Private Limited, Nagpur, Maharashtra, given standardized (20% - 30% saponins) *C. nurvala* bark extract, and S.A. Herbal Bioactive, Mumbai, Maharashtra, provided standardized (Tannins NLT 5% / 18:1) *P. marsupium* bark extract.

In-vitro anti-inflammatory activity

An *in vitro* technique was used to investigate the anti-inflammatory effects of PMSBE and CNSBE, with the presumption that the activation of lysosomal enzymes during inflammation causes any disarray. Acute inflammation is the most common disorder among them because of their extracellular function. By inhibiting these chemical mediators or stabilizing the lysosomal membrane, the ability of experimental compounds may be measured. The prevention of hypotonicity-induced membrane lysis of human red blood cells (HRBC) was used to test anti-inflammatory properties since the membranes of human red blood cells and lysosomal membrane components are similar. For this treatment, blood was obtained from a healthy individual who had not taken any anti-inflammatory drug in the past 15 days. Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) was filtered and centrifuged at 3000 rpm in an equal quantity. Plasma was isolated and stored with caution. Before being suspended in a 10% solution, the sealed blood corpuscles are cleaned in a 0.9% saline solution. Aliquots of plant extract were prepared with distilled water for concentrations of 250 µg/mL. Every concentration received 1 mL phosphate buffer, 2 mL hyposaline (hyposaline), and 0.5 mL HRBC suspension. After 30 mins of incubation at 37°C ± 1°C, the contents were centrifuged at 3000 rpm for 20 mins. The hemoglobin content of the supernatant solution was measured using spectrophotometry at 560 nm with diclofenac sodium as a reference standard. Aside from the extract, a control was also produced. The level hemolysis was calculated assuming that the control sample had 100% hemolysis.[8] Using the formula, the percentage of HRBC membrane stabilization by plant extract was determined:

$$\% \text{ Protection} = 100 - \frac{\text{OD of Drug treated sample}}{\text{OD of Control}} \times 100$$

Statistical analysis

Three times the procedure was carried out. The data are provided in the form of a mean standard deviation (SD). For the statistical equations, Minitab® version 17 was used. For pharmacological procedures, the unpaired Student t-test (two-tailed) was used to assess the disparity between the monitoring and examined classes.

RESULTS

In vitro anti-inflammatory activity showed that both extracts, alone and in addition, had significant activity as compared to the standard diclofenac sodium (74.49%). PMSBE provided 42.88% membrane stabilization /protection in HRBCs at a concentration of 250 µg/mL, while CNSBE provided 38.26% membrane stabilization/protection in HRBCs. The anti-inflammatory activity was improved to 59.52% when the extracts PMSBE and CNSBE were measured together (at a concentration of 250 µg/mL each) (**Table 1**). When all extracts were used at the same time, the anti-inflammatory value increased from 16% to 21%. Anti-inflammatory activity was measured using hypotonicity suppression and heat-induced red blood cell membrane lysis. The presence of alkaloid, phenolic, and flavonoid compounds, all of which have strong anti-oxidant properties, may clarify these results.

Table 1: *In vitro* anti-inflammatory potential of *Pterocarpus marsupium* standardized bark extract and *Crataeva nurvala* standardized bark extract combination.

Treatment	Concentration (µg/mL)	Absorbance (560 nm)	% Protection[#]
Control	-	0.541 ± 0.003	-
Pterocarpus marsupium bark standardized extract	250	0.309 ± 0.005***[a]	42.88
Crataeva nurvala bark standardized extract	250	0.334 ± 0.002***[a]	38.26
Pterocarpus marsupium bark standardized extract + *Crataeva nurvala* bark standardized extract	250 + 250	0.219 ± 0.002***[a]	59.52
Diclofenac sodium	100	0.138 ± 0.001***[a]	74.49

All values represent mean ± SD of n = 3;***p<0.001 with respect to the control group. [a]Determined as compared with the control group (solution of 0.9% sodium chloride) using the above formula. [#]% protection offered by the extract or standard refers to the prevention of hypotonicity-induced HRBC membrane lysis.

DISCUSSION

Enzymes are produced as lysosomal components are lyzed during inflammation, aggravating a number of conditions. Anti-inflammatory drugs act by inhibiting the release of lysosomal enzymes or stabilizing the membranes around the lysosomes. The capacity of thorn extract to suppress hypotonicity-induced HRBC membrane lysis was used to predict anti-inflammatory properties since the membranes of human red blood cells and lysosomal membranes are very similar. The extract's strong phenolic and flavonoid content worked together to balance the HRBC membrane in a synergistic way. The active components of classes such as alkaloids or flavonoids are thought to express a key role in suppressing inflammation by inhibiting inflammatory enzymes such as COX, LOX, PGDH, and PGS.[8]

P. marsupium extract was shown to lower prostaglandin E_2 (PGE_2) levels in human peripheral blood, likely by inhibiting the inflammatory mediator COX-2.[9] The active phytoconstituents have been discovered to play a critical function in mediating the effect. The plant contains protein, pentosan, pterosupin, pseudobaptigenin. liquiritigenin, isoliquiritigenin, garbanzol, 5-deoxykaempferol, *p*-hydroxybenzaldehyde, beta-eudesmol, erythrodirol-3-monoacetate, pterostilbene, l-epicatechin, marsupol, carpusin, propterol, propterol B, marsupinol, irisolidone-7-O-α-L-rhamnopyranoside, 6-hydroxy-2-(4-hydroxybenzyl)-benzofuran-7-C-β-D-glucopyranoside, 3-(α-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3*H*)-furanone-7-C-β-D-glucopyranoside, 2-hydroxy-2-*p*-hydroxybenzyl-3(2*H*)-6-hydroxybenzofuranone-7-C-β-D-glucopyranoside, 8-(C-β-D-glucopyranosyl)-7,3′,4′-trihydroxyflavone, 1,2-bis(2,4-dihydroxy,3-C-glucopyranosyl), 6-hydroxy-3,5,7,4′-tetramethoxyflavone-6-O-rhamnopyranoside, 2,4',6-trihydroxy-4-methoxybenzo(b)furan-3(2*H*)one, 1,3-bis(4-hydroxyphenyl)propan-2-ol, 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propan-2-ol, and 6-hydroxy-7-*O*-methyl-3-(3-hydroxy-4-*O*-methyl benzyl) chroman-4-one.[6]

Lupeol, extracted from the stem bark of *C. nurvala*, has anti-inflammatory properties. Lupeol linoleate and indomethacin reduce paw swelling by 39%, 58%, and 35%, respectively, in adjuvant arthritis.[10] Phytoconstituents have been discovered to play a critical role in mediating pharmacological activity expression. The chief chemical constituents include triterpenoids, lupeol, varunol, rutin, quercentine, isoquercetine, ceryl alcohol, triacontane, triacontanol, friedeline, betulenic acid, diosgenin, cetyl alcohol, lenoleic acid, choline, lauric acid, cadabicine, stearic acid, diosgenin, undecyclic acid, oleic acid, and gluco-capparin.[7]

CONCLUSION

The mixture of *Pterocarpus marsupium* standardized bark extract and *Crataeva nurvala* standardized bark extract had a significant anti-inflammatory effect *in vitro* at a concentration of 250 µg/mL. Flavonoids, phenols, and alkaloids, which are present in plant products, play a crucial role in mediating anti-inflammatory activity by inhibiting multiple inflammatory enzymes. This study will pave the way for further studies into the discovery of novel herbal formulations for the treatment of acute and chronic inflammation.

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CONFLICT OF INTEREST

The authors declare no Conflict of Interest regarding the publication of the article.

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REFERENCES

1. Mahapatra DK, Dadure KM, Shivhare RS. Exploring the Site-Specific Influence of Hydroxyl group in Ring-B of Murrayanine-Chalcone on Edema Reducing Potential. MOJ Drug Design Devel Ther, 2018; 2(4): 191-4.
2. Mahapatra DK, Shivhare RS, Das M. Edema Reducing Prospective of Murrayanine based

Chalcone Compound as Emerging NSAID Agent. Innoriginal Int J Sci, 2019; 6(3): 1-4.
3. Mahapatra DK, Dadure KM, Shivhare RS. Edema Reducing Potentials of Some Emerging Schiff's bases of Murrayanine. MOJ Bioorg Org Chem, 2018; 2(4): 172-5.
4. Mahapatra DK, Thote LT, Dhabarde DM. The emerging anti-inflammatory potentials of some novel Schiff's base derivatives of 3,4-dioxymethylene acetophenone. J Appl Pharm Sci Res, 2018; 1(3): 1-4.
5. Mahapatra DK, Dadure KM, Haldar AGM. Exploring the anti-inflammatory potentials of N-((5-(((1,3-dioxoisoindolin-2-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide. Drug Discov, 2018; 12: 42-5.
6. Gairola S, Gupta V, Singh B, Maithani M, Bansal P. Phytochemistry and pharmacological activities of Pterocarpus marsupium: a review. Int Res J Pharm, 2010; 1(1): 100-4.
7. Nigamanand B, Suresh C, Chandra TR, Deep KG, Srishti D. Varun (Crataeva nurvala Buch-Ham): a critical review w.s.r. to urinary tract disorder. Int J Ayur Pharm Res, 2016; 4(4): 49-52.
8. Kamble MA, Mahapatra DK, Dhabarde DM, Ingole AR. Pharmacognostic and pharmacological studies of Bombax ceiba thorn extract. J Pharm Pharmacog Res, 2017; 5(1): 40-54.
9. Sanders A, Smit HF, Garssen J, Faber J, Hoijer MA. Pterocarpus marsupium extract exhibits antiinflammatory activity in human subjects. Planta Med, 2005; 71(5): 387-92.
10. Vashist S, Choudhary M, Shammi Rajpal DS, Budhwar V. Varuna (Buch. Ham.): A brief review on Phytochemistry, Crataeva nurvala Pharmacological profile and uses in various ailments. Asian J Pharm Pharmacol, 2020; 6(2): 119-25.