



Investigating the Synergistic *In-vitro* Anti-inflammatory Potentials of Standardized Fruit Extract of *Eugenia jambolana* and Standardized Fruit Extract *Piper nigrum* Combinations

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ABSTRACT

After searching PubMed, Google Scholar, Scopus, and other pharmaceutical databases, it was discovered that no combined anti-inflammatory viewpoints of *Eugenia jambolana* fruit extract and *Piper nigrum* fruit extract have been published in an *in vitro* technique (human red blood cells, HRBC). To investigate the synergistic activity, the *E. jambolana* standardized fruit extract (EJSFE) and *P. nigrum* standardized fruit extract (PNSFE) were screened together. EJSFE gave 27.23 percent membrane stabilization/protection in HRBCs at a concentration of 250 µg/mL, whereas PNSFE supplied 32.31 percent membrane stabilization/protection in HRBCs. The anti-inflammatory impact was raised to 58.63 percent when the extracts EJSFE and PNSFE were evaluated combined (at a concentration of 250 µg/mL each). When both extracts were administered at the same time, the anti-inflammatory effect increased by 25 percent to 30 percent. Flavonoids, phenols, tannins, and alkaloids are phytoconstituents present in plant material that play a significant role in mediating anti-inflammatory effects by blocking several inflammatory enzymes. The extract's strong phenolic, tannin, and flavonoid content worked together to stabilize the HRBC membrane in a synergistic way. This paper will pave the way for future research into the creation of novel herbal formulations for the treatment of acute and chronic inflammation.

Key Words: *Eugenia jambolana*, *Piper nigrum*, Extract, anti-inflammatory, HRBC Method, Phytoconstituents

INTRODUCTION

Inflammation is a process in which white blood cells and a variety of important chemical mediators are generated in order to protect us against dangerous organisms such as bacteria. While the mechanism is necessary for life, it causes agonizing pain and agony when it is aggravated. ^[1] Inflammatory diseases, such as inflammation, cause individuals to feel far more than they should and assault the body's own tissue. As a consequence, anti-inflammatory medications are seldom needed to alleviate pain and the resulting responses. ^[2] Many biochemical processes in the inflammatory process 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 production of leukotrienes, which are essential in acute inflammation. ^[3] A variety of anti-inflammatory medications, both steroidal and non-steroidal, with properties of inhibiting these multiple mediators have attracted pharmacotherapeutic

interest, but they have not been widely used for a variety of reasons, including impaired pharmacokinetics, unintended side effects, and so on. ^[4] In order to generate an ideal medicine, attempts are made on a regular basis to tackle these anti-inflammatory-related challenges using a number of pragmatic ways. ^[5]

Eugenia jambolana is known to possess diverse phytochemicals, most of which are observed to be of health benefits. The leaves are known to contain betulinic acid, β -sitosterol, crategolic (maslinic) acid, mycaminose, n-nonacosane, n-hepatcosane, noctacosanol, n-hentriacontane, n-dotricontanol, n-triacontanol, myricetin, quercetin, myricetin 3-O-(4"-acetyl)- α -L-rhamnopyranosides, and myricitrin. The essential oil from the leaves is shown to contain the phytochemicals α -terpeneol, pinocarveol, eucarvone, myrtenol, α -myrtenal, muurolol, geranyl acetone, cineole, pinocarvone, and α -cadinol. The stem bark is reported to possess friedelan-3- α -ol, friedelin, β -sitosterol, betulinic acid, β -sitosterol-*D*-

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glucoside, kaempferol, ellagic acid, gallic acid, ellagitannin, gallotannin, and myricetine which contribute to anti-bacterial activity, anti-fungal activity, anti-viral activity, free radical scavenging, free radical scavenging, anti-inflammatory effects, gastroprotective effects, hepatoprotective effects, anti-diabetic activities, hypolipidemic effect, cardioprotective effects, anti-diarrheal effects, anti-fertility activity, anti-allergic effects, anti-pyretic effects, neuropsychopharmacological effects, anti-neoplastic effects, chemopreventive effects, radioprotective effects, anti-clastogenic effects, etc. [6]

The phytochemical investigations of *P. nigrum* revealed that it contains a variety of phytochemicals. Piperine was the first pharmacologically active compound isolated from different members of the Piperaceae family. Many investigators isolated different types of compounds viz flavonoids, phenolics, amides, alkaloids, lignans, steroids, terpenes, neolignans, chalcones, etc. Some of the compounds are dihydro-piperidine, brachyamide B, *N*-trans-feruloyltryamine, (2*E*,4*E*)-*N*-eicosadienoyl-periperidine, guineensine, *N*-formylpiperidine, (2*E*,4*E*)-*N*-isobutyldecadienamid, pentadienoyl, tricholein, isobutyl-eicosadienamide, isobutyl-eicosatrienamide, trichostachine, piperamide, isobutyl-octadienamide, piperettine, piperamine, pipericide, piperolein B, piperine, sarmentosine, sarmentine, and retrofractamide A which contributes to anti-hypertensive activity, anti-asthmatic activity, cognitive action, fertility activity, anti-microbial activity, anti-oxidant activity, anti-cancer activity, anti-inflammatory activity, hepatoprotective activity, anti-diarrheal activity, digestive activity, anti-depressant activity, immunomodulatory activity, anti-convulsant activity, analgesic activity, etc. [7]

After searching PubMed, Google Scholar, Scopus, and other pharmaceutical databases, it was discovered that no combined anti-inflammatory viewpoints of *E. jambolana* fruit extract and *P. nigrum* fruit extract have been recorded in an *in vitro* technique (human red blood cells method). To investigate the synergistic activity, the *E. jambolana* standardized fruit extract (EJSFE) and *P. nigrum* standardized fruit extract (PNSFE) were screened together.

MATERIALS AND METHODS

Chemicals

The standard diclofenac sodium was given by Bangalore-based Sigma Aldrich® Ltd. The reagents, consumables, and chemicals for this investigation were bought from HiMedia® India Pvt. Ltd., Mumbai, via a local distributor. A double-distilled water apparatus (Borosil®, India) was used in the experiment.

Instruments

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

Plant materials

S. A. Herbal Bioactive, Mumbai, Maharashtra, offered standardized *E. jambolana* fruit extract (Tannins NLT 10%; 2.5 percent - 5 percent Bitters) and standardized *P. nigrum* fruit extract (5 percent - 95 percent Piperine).

In-vitro anti-inflammatory activity

The anti-inflammatory effects of EJSFE and PNSFE were investigated utilizing an *in vitro* technique based on the hypothesis that the release of lysosomal enzymes during inflammation causes some disarray. Acute inflammation is the most prevalent ailment among them because of its extracellular activity. By blocking these chemical mediators or stabilizing the lysosomal membrane, the ability of experimental drugs may be calculated. The prevention of hypotonicity-induced membrane lysis in human red blood cells (HRBC) was utilized to investigate anti-inflammatory effects since the membranes of human red blood cells and lysosomal membrane components are similar. For this technique, blood was obtained from a healthy individual who had not used any anti-inflammatory medication in the preceding 15 days. Alsever's solution (2 percent dextrose, 0.8 percent sodium citrate, 0.5 percent citric acid, 0.42 percent sodium chloride) was diluted and centrifuged at 3000 rpm in an identical quantity. Plasma was separated and preserved with care. Before being suspended in a 10% solution, the sealed blood corpuscles are cleaned in a 0.9 percent saline solution. Aliquots of plant extract were produced with distilled water at concentrations of 250 µg/mL. Each concentration received 1 mL phosphate buffer, 2 mL hyposaline (hyposaline), and 0.5 mL HRBC solution. After 30 minutes of incubation at 37°C ± 1°C, the contents were centrifuged at 3000 rpm for 20 minutes. The hemoglobin concentration of the supernatant solution was measured using spectrophotometry at 560 nm with diclofenac sodium as a reference standard. Aside from the extract, control was also made. The percentage hemolysis was calculated assuming that the test group had 100% hemolysis. [8] The percentage of HRBC membrane stabilization by plant extract was calculated using the formula:

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

Statistical analysis

Three times the experiment was carried out. The data was

given in the form of a mean standard deviation (MSD). For the mathematical computations, Minitab® version 17 was employed. For pharmaceutical procedures, the unpaired Student t-test (two-tailed) was used to evaluate the disparity between the monitoring and studied groups.

RESULTS

In vitro anti-inflammatory efficacy demonstrated that all extracts, alone and in combination, exhibited significant action when compared to the standard medication diclofenac sodium (75.69 percent). EJSFE gave 27.23 percent membrane stabilization/protection in HRBCs at a concentration of 250 µg/mL, whereas PNSFE supplied 32.31 percent membrane stabilization/protection in HRBCs. The anti-inflammatory impact was raised to 58.63 percent when the extracts EJSFE and PNSFE were evaluated combined (at a concentration of 250 µg/mL each) (Table 1). When both extracts were administered at the same time, the anti-inflammatory effect increased by 25 percent to 30 percent.

Table 1: *In vitro* anti-inflammatory potential of *Eugenia jambolana* fruit standardized extract and *Piper nigrum* fruit standardized extract combination.

Treatment	Concentration (µg/mL)	Absorbance (560 nm)	% Protection [#]
Control	-	0.551 ± 0.003	-
<i>Eugenia jambolana</i> fruit standardized extract (EJSFE)	250	0.401 ± 0.004 ^{***a}	27.23
<i>Piper nigrum</i> fruit standardized extract (PNFSE)	250	0.373 ± 0.002 ^{***a}	32.31
EJSFE + PNFSE	250 + 250	0.228 ± 0.004 ^{***a}	58.63
Diclofenac sodium	100	0.134 ± 0.003 ^{***a}	75.69

All values represent mean ± SD of n = 3; ***p < 0.001 with respect to the control group. ^aDetermined 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.

CONCLUSION

The combination of *E. jambolana* standardized fruit extract and *P. nigrum* standardized fruit extract, at a dosage of 250 µg/mL, exhibited a significant anti-inflammatory effect *in vitro*. Flavonoids, tannins, phenols, and alkaloids, among other phytoconstituents present in plant material, play a vital role in mediating anti-inflammatory effects by blocking inflammatory enzymes. This research will offer up new research opportunities for the creation of novel herbal formulations for the treatment of acute and chronic inflammation.

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DISCUSSION

The anti-inflammatory effect was measured using hypotonicity suppression and heat-induced red blood cell membrane lysis. The presence of alkaloid, phenolic, tannin, and flavonoid chemicals, all of which have strong anti-oxidant properties, might explain these results. Enzymes are produced when lysosomal components are lysed during inflammation, exacerbating a range of illnesses. Anti-inflammatory medications operate by blocking the release of lysosomal enzymes or stabilizing the membranes surrounding the lysosomes. The capacity of thorn extract to suppress hypotonicity-induced HRBC membrane lysis was utilized to assess anti-inflammatory capabilities since the membranes of human red blood cells and lysosomal membranes are so similar. The extract's strong phenolic and flavonoid content worked together to stabilize the HRBC membrane in a synergistic way. Flavonoids, for example, are considered to play a key role in decreasing inflammation by inhibiting inflammatory enzymes such as COX, LOX, PGDH, and PGs.^[8]

CONFLICT OF INTEREST

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

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