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Antipyretic Activity of β -Sitosterol Isolated from Leaves of *Oxalis Corniculata* LINN.

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ABSTRACT

Oxalis corniculata Linn. (Oxalidaceae) is one of the important medicinal plants used traditionally for the treatment of fever, pain and inflammation. The present study was aimed to screen antipyretic activity of petroleum ether, chloroform, ethyl acetate, methanol extracts and β-sitosterol isolated from petroleum ether extract of *Oxalis corniculata* Linn. The leaves of *Oxalis corniculata* Linn. was used for successive extraction with increasing polarity solvents. Petroleum ether extract was selected for activity-guided fractionation to isolate β-sitosterol due to its better efficacy than other extracts. Antipyretic activity was done by yeast induced pyrexia method. All the extracts were screened at the dose of 100 mg/kg, i.p. and isolated β-sitosterol was screened at the dose of 10 and 20 mg/kg. The result showed that after five hour of petroleum ether extract (100 mg/kg) administration significant inhibition of pyrexia up to 89.5% was observed. Isolated β-sitosterol 10 and 20 mg/kg, i.p. showed promising antipyretic activity with inhibition of pyrexia by 91.23 and 94.41% respectively. Results are compared with standard drug paracetamol (50 mg/kg i.p.). Thus the present pharmacological screening provides support for the folklore claim of antipyretic activity of *Oxalis corniculata* Linn.

Keywords: *Oxalis corniculata* Linn., Brewer's yeast, Pyrexia, β-sitosterol.

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INTRODUCTION

Oxalis corniculata Linn. a sub-tropical plant is native of India, commonly known as changeri in marathi. This plant is a delicate-appearing, low growing, herbaceous and abundantly distributed in damp shady places and lawns nearly all regions throughout warmer part of Maharashtra.[1] It is locally used in treating various ailments. It is rich in niacin, vitamin C and β-carotene.[2] The juice of the plant is given in jaundice and in stomach troubles.[3] The leaf extract of the plant mixed with butter is applied to muscular swellings and pimples.[4] *Oxalis corniculata* Linn. is also used as antiseptic, refrigerant, diaphoretic, diuretic and anti diabetic.[5] It is used as complementary medicine in wound healing, anemia, dyspepsia, cancer, piles, dementia and convulsions.[6,7] Other alternative uses are; anthelminthic, anti-inflammatory, astringent, depurative, diuretic, stomachic and styptic. It is also used in the treatment of urinary tract infections, influenza, fever, traumatic injuries and sprains.[8] It was also reported that plant has hypoglycemic, antipsychotic, nervous system stimulant and have chronotropic and inotropic effect.[9] Chemical characterization showed the presence of glyoxylic acid, oxalic acid, pyruvic acid, vitexin and isovitexin, vitexin-2-O-beta-D-glucopyranoside, neutral lipids, glycolipids; vitamin C; phospholipids; fatty acids, 18:2, 18:3, 16:0; saturated (C10-C14) acids; alpha and beta tocopherols. Herbal medicine is a major component in all traditional medicine systems and a common element in Ayurvedic, Unani, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine. As per literature herbal medicines are assuming greater importance in the primary health care of individuals and communities in rural areas. Plants and their derivatives are invaluable source of therapeutic agents to treat various disorders. Herbal products are often perceived as safe because they are natural and having less side effect. Considerable efforts have been directed towards the development of natural products from various plant sources.[10] Substantial number of drugs are developed from plants which are active against various diseases and disorders. Nowadays research involve the isolation of the active constituent (chemical compound) found in a particular medicinal plant and its subsequent modification or synthesis.[11]

Figure 1: Leaves of *Oxalis corniculata* Linn.

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. [12] Cytokines, interleukin, interferon and Tumor Necrosis Factor-α (TNF-α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature[13]. Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance heat generation and loss[14]. Synthetic antipyretic drugs like NSAID at high doses reported serious common side effects such as hepatotoxicity, hyperacidity, gastritis etc hence there is a need to focus on the scientific exploration of potential herbal drugs having fewer side effects.[15, 16] Present study was designed to evaluate potential of various leaf extracts and β-sitosterol isolated from leaves of Oxalis *Corniculata* Linn. in the treatment of brewer's yeast induced pyrexia.

MATERIALS AND METHOD

Plant material

Leaves of *Oxalis corniculata* Linn. was collected from the Loni (Shirdi) periphery in the Ahmednagar district of Maharashtra. It was authenticated by Dr. Diwakar, Joint Director of Botanical Survey of India, Pune. A voucher specimen (DSOC001) has been deposited in the herbarium section of the department of Pharmacognosy, PRCOP, Loni, for future reference. The leaves (5.5 kg) were air dried and pulverized using a mechanical grinder.

Extraction and isolation

Dried and powdered leaves (750 g) of the plant were extracted successively with various solvents viz. petroleum ether, chloroform, ethyl acetate and methanol in soxhlet extractor. The mark left was extracted using water as solvent. Extracts were concentrated by vacuum distillation and then dried in open air to produce the respective extracts. All the extracts were vacuum dried to obtain petroleum ether extract (7.24%), chloroform extract (6.034%), ethyl acetate extract (4.48%) and methanol extract (8.54%) respectively. The petroleum ether extract (5.0 g) was subjected to column chromatography on silica gel column. The column was eluted by gradient elution method using hexane and ethyl acetate with increasing polarity. Fractions of 97–105 eluted with 3% ethyl acetate in hexane were found similar on TLC with a major spot and a minor spot. The concentrated solution was kept for 24 h at room temperature to precipitate needle-shaped crystals. The resultant crystals were separated from liquid phase and washed successively with pure hexane and 1, 2 and 5% ethyl acetate in hexane. The white substance (30 mg) was re-crystallized from methanol and it showed a single spot on TLC (Rf 0.47, ethyl acetate/ hexane, 2:8). The isolated compound was identified by studying its melting point, UV, FTIR, MS, ^{1}HNMR and ^{13}CNMR spectroscopy.

Animals

Wistar Albino rats (150–200 g) weighing 150–200 g were housed under standard laboratory conditions, in groups of six each and used for antipyretic activity. The animal had free access to water and food ad libitum. The ethical committee of the institute approved the protocol of the study having IAEC no. 448/01/c/CPCSEA/13-14/19

Evaluation of antipyretic activity

Animals of constant rectal temperature for a week were selected for the experiment. The antipyretic activity of the extracts and isolated β-sitosterol was evaluated based on brewer's yeast induced pyrexia in rats. Anti-pyretic activity was measured by slightly modifying the method described with Albino rats by Venkatesh *et al.*[17] Before the experiments, for 18 hours, pyrexia was induced by subcutaneously injecting 10ml/kg brewer's yeast into the animals dorsum region. Eighteen hour after the injection, digital thermometer was used to check rectal temperature of each rat by inserting probe in to rectum. Only rats showing an increase in temperature of at least 0.7°C were used for experiment. Wistar rats of either sex were divided into eight groups of six animals each. Group I was treated with normal saline as a control. Paracetamol (50 mg/kg i.p.) given to group II served as a standard. The animals of the III to VI groups were treated with petroleum ether (PEOC), chloroform (CFOC), ethyl acetate (EAOC) and methanol (MEOC) extracts (100 mg/kg, i.p. each) respectively. Group VII and VII treated with isolated β-sitosterol 10 and 20 mg/kg, i.p. respectively. The rectal temperatures were recorded at 1, 3 and 5 hour after treatment[18]. Percentage reduction in rectal temperature was calculated by following formula

$$\text{Percentage reduction in rectal temperature} = \frac{Y-X}{Y-Z} \times 100$$

Where Z= Initial rectal temperature ^{0}C, Y = Rectal temperature 18 hour after yeast administration; X = Rectal temperature after extract administration.

Statistical analysis

All data were expressed as mean ± SEM. The statistical analysis of all the observations was carried out using one-way ANOVA followed by multiple comparison test of Tukey–Kramer, where necessary. $P<0.05$ was considered as significant compared with the control group.

RESULTS AND DISCUSSION

Identification of isolated compound

The isolated compound was a crystalline solid with melting point 136 –138°C. It showed single

spot on TLC with Rf 0.47 (ethyl acetate/hexane, 2:8). The isolated compound gave positive Libermann–Burchard test which shows that the compound was a sterol. The mass spectrum showed M at m/z at 414, corresponding to the molecular formula ($C_{29}H_{50}O$). The IR spectrum showed the occurrence of – OH group (3,434 cm^{-1}). The result showed two singlets at d 1.00 and 0.67 that were assigned to the methyl group of C-19 and C-18, respectively in 1H-NMR spectrum. The doublets at d 0.92 (d, J = 6.1 Hz), 0.81(d, J = 6.9 Hz), 0.83 (d, J = 6.9 Hz) and 0.84 (t, J = 7.3 Hz) accounted for the methyl group at C-21, C-26 and C-27. Signals at d 5.35 in 1H-NMR can account for an olefinic proton at C-6. Other multiplet at d 3.52 equivalents to a singlet proton was assigned for the proton of C-3. The low field signal may be due to the attachment of b-OH group at the C-3 carbon. Thus, the assignment of hydroxyl group at C-3 and the double bond at C-5 were assigned accordingly. Three multiplets equivalent to two protons each appeared at d 1.83, 2.00 and 2.27 and were assigned to three CH_2 groups. The remaining protons appeared as multiplets at d 1.05–1.65. From all the spectral analysis and Co-TLC with authentic sample, the isolated compound was identified as β-sitosterol (Figure 3). ^{13}CNMR gave signal at 140.8 and 121.7 ppm for C_5=C_6 double bond, respectively, 71.8 for C3 b-hydroxyl group, and 19.4 and 11.9 for the angular methyl carbon atoms for C_{19} and C_{18}, respectively.

Antipyretic activity

All the extracts and isolated β-sitosterol showed antipyretic activity as compared to control group (Table 1). After eighteen hour administration of Brewer's yeast injection the experimental rats showed a mean increase in rectal temperature up to 2.52°C. In control group after yeast administration the rectal temperature was raised up 39.30°C from normal body temperature 36.82°C. Second group with paracetamol treatment showed percentage inhibition of pyrexia up to 51.54, 70.05 and 96.06% after 1, 3 and 5 hour respectively. In third group PEOC administration inhibited the pyrexia up to 62.18 and 89.5% after 3 and 5 hour respectively. With chloroform and ethyl acetate extract percentage pyrexia reduction was up to 62.29 and 61.38% after five hour respectively. Group seven and eight treated with β-sitosterol 10 and 20 mg/kg inhibited the pyrexia up to 91.23 and 94.41% after five hour of administration respectively. PEOC and β-sitosterol showed significant antipyretic activity throughout the observation period as compared to standard drug paracetamol (Figure 2). As per literature survey the available antipyretics have toxic effect to the various body organs hence there is need to search herbal remedies with potent antipyretic activity.[19] In view of this present study was designed to screen antipyretic activity of the different extract of *Oxalis corniculata* Linn. and isolated β-sitosterol from petroleum ether extract. The body's ability to maintain a natural balance of COX-1 and COX -2 that regulate inflammatory

response play a crucial role in supporting various systems.[20, 21] The results showed that all the extracts possess antipyretic activity but petroleum ether extract showed significant antipyretic effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats and its effect is comparable to that of the standard antipyretic drug paracetamol. Hence petroleum ether extract was selected for isolation and characterization of the active constituent. The phytochemical investigation showed that petroleum ether extract contains β-sitosterol which was isolated by TLC techniques and characterized by studying its melting point, UV, FTIR, MS, ^{1}HNMR and ^{13}CNMR spectroscopy. Moreover, the statistical analysis with two-way ANOVA showed that the all extract of *Oxalis corniculata* Linn. decreased yeast elevated body temperature in a short span of time, when compared with control group. The isolated fraction i.e. β-sitosterol was more effective than the PEOC as compared to standard drug paracetamol. The β-sitosterol is a plasminogen activator promotes the formation of essential polyunsaturated fatty acids from linoleic acid, required for prostaglandin and leukotriene synthesis.[22,23] It was evident from the study that the observed antipyretic effects of the extract were similar in both magnitude and time course. The present results showed that the petroleum ether extract of *Oxalis corniculata* Linn. (PEOC) and β-sitosterol possess a significant antipyretic effect in yeast-provoked elevation of body temperature in rats and its effect is comparable to that of Paracetamol (Standard drug).

Table 1: Effect of different extracts and isolated β-sitosterol on yeast induced pyrexia

Treatment	Normal rectal temperature before yeast administration	Rectal temperature after 18 hrs of yeast administration(°C)	Rectal temperature and percentage reduction in rectal temperature at different time intervals (°C) Time after treatment (hr)		
			01 hr	03 hr	05 hr
Control	36.82 ± 1.66	39.30 ± 0.37	38.94 ± 0.35 (14.52%)	38.68 ± 0.50 (25.00%)	38.45 ± 0.33 (34.27%)
Paracetamol (50 mg/kg, i.p.)	35.58 ± 1.11	38.82 ± 0.63	37.15 ± 0.96 (51.54%)	36.55 ± 0.84*(70.06%)	35.69 ± 1.19** (96.6%)
PEOC (100 mg/kg, i.p.)	36.74 ± 0.97	39.12 ± 0.40	38.34 ± 0.54 (32.77%)	37.64 ± 0.43 (62.18%)	36.99 ± 0.65* (89.5%)
CFOC (100 mg/kg, i.p.)	36.44 ± 1.40	38.80 ± 0.58	38.2± 0.79 (25.42%)	37.64 ± 0.84 (49.15%)	37.33± 01.07 (62.29 %)
EAOC (100 mg/kg, i.p.)	36.20 ± 1.40	38.66 ± 0.81	38.15 ± 0.89 (20.73%)	37.6 ± 0.66 (43.09%)	37.15 ± 0.91 (61.38%)
MEOC (100 mg/kg, i.p.)	36.56 ± 1.52	39.00 ± 0.32	38.54 ± 0.94 (18.85%)	37.56 ± 0.50 (59.02%)	37.28 ± 0.77* (70.49%)
β-sitosterol (10 mg/kg, i.p.)	36.70 ± 1.40	38.98 ± 0.77	38.26 ± 0.81 (31.58%)	37.51 ± 0.559 (64.47%)	36.9 ± 0.84** (91.23%)
β-sitosterol (20 mg/kg, i.p.)	36.65 ± 0.45	38.8 ± 0.57	37.85 ± 0.96 (44.18%)	37.23 ± 0.84* (73.02%)	36.77± 0.56** (94.41%)

Values are expressed as mean ± S.E.M; n=6; * P< 0.05 and **P< 0.01; as compared to the control.

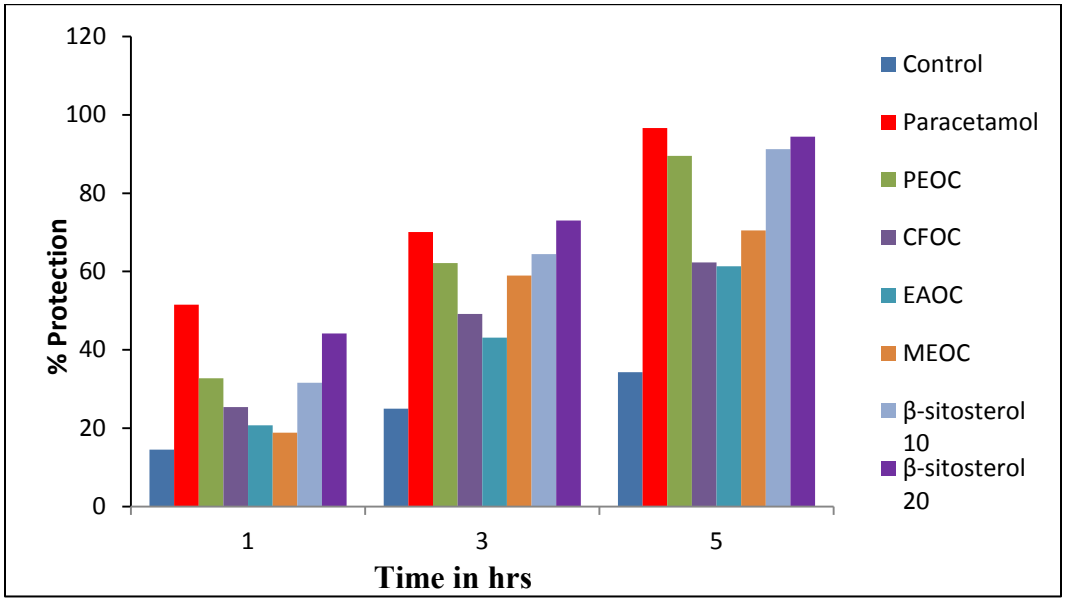

Figure 2: Percentage protection on yeast induced pyrexia of different extracts and isolated β-sitosterol

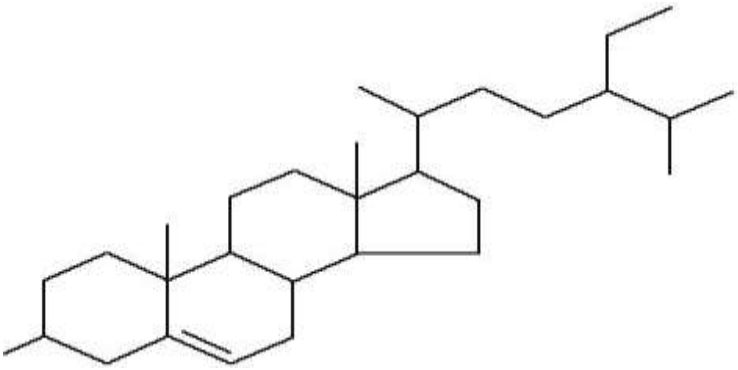

Figure 3: Structure of β-sitosterol

CONCLUSION

Hence we conclude that the *Oxalis corniculata* Linn. possess significant antipyretic activity by brewer's yeast induced method. Petroleum ether extract contains β-sitosterol as one of the major compound. The antipyretic activity of the plant can be assigned to presence of β-sitosterol. Thus the present pharmacological screening provides evidence for the folklore claim of antipyretic activity of *Oxalis corniculata* Linn.

REFERENCE

1. Ackola KJ, Mwangi JW. Smooth muscle relaxant, cardio relaxant, hypotensive activity of *Oxalis corniculata*. International Journal of Pharmacognosy, 1995; 33: 247.
2. Anonymous. In, The Wealth of India. A dictionary of Indian Raw Material and Industrial Products, CSIR, New Delhi; 1958: 198.

3. Asongalem EA, Foyet HS, Ngogang J, Folefoc GN, Dimo T, Kamtchouing P. Analgesic and anti-inflammatory activities of Erigeron floribundus. J Ethnopharmacol, 2004; 91: 301 – 308.
4. Brune K, Alpermann H. Non-acidic inhibition of prostaglandin production, carrageenine oedema and yeast fever. Ag action, 1983; 13: 360 – 363.
5. Dray A, Perkin M. Bradykinin and inflammatory pain. Trends Neurosci, 1993; 16: 99–104.
6. Gaitonde BB, Jogalekar SN, Kulakarni HJ, Nabar SD. Anti-inflammatory activity of *Oxalis corniculata* in carrageenan induced paw oedema in rats. J Res Indian medicine, 1977; 12: 12.
7. Garcia MD, Fernandez MA, Alvarez A, Saenz MT. Antino-ciceptive and anti-inflammatory effect of the aqueous extract from leaves of Pimenta racemosa var. ozua (Mirtaceae). J Ethanopharmacol, 2004; 91: 2973.
8. Kirtikar KR, Basu BD Indian medicinal plants. Vol VII, New Delhi, Sri Satguru Publications ; 2000: 2110–2113.
9. Hazeena Begum V and Velavan S. Lipids regulating activity of *Asparagus racemosus* root in young and aged rats. Indian J Gerontology, 2011; 25(3): 273-285.
10. Ignacimuthu. S, Ayyanar M. Herbal medicines for wound healing among tribal people in Southern India: Ethno botanical and Scientific evidences. Int J Applied Research in Natural Products, 2009; 2 (3): 29-42.
11. Rajani GP, Gupta D, Sowjanya K, Sahithi B. Screening of antipyretic activity of aerial parts of *Nelumbo nucifera* gaertn in yeast induced pyrexia . Pharmacologyonline, 2011; 1: 1120-1124.
12. Duraisankar M. Ravichandran V. Antipyretic Potential of Polyherbal Ayurvedic Products. Asian Journal Pharmaceutical and Clinical Research, 2012; 5 (2): 146 – 150.
13. Jayalaksmi S, Ghosh AK, Birendra S, Singla RK. Preliminary Investigation of Antipyretic Activity of Trinpanchmool Extracts. Int J Phytomedicine, 2011; 147-150.
14. Gupta M, Shaw BP, Mukerjee A. Studies on Antipyretic Analgesic and Ulcerogenic Activity of Polyherbal Preparation in Rats and Mice. Intl. Journal of Pharmacology, 2008; 4(2): 88-94.
15. Ighodaro I, Ozolua RI, Okpo SO, Obasuyi O. Antipyretic and analgesic effects of the aqueous extract of the Fruit pulp of *Hunteria umbellata* K Schum (Apocynaceae). Tropical J Pharma Res 2009; 8(4): 331-336.
16. Periyasamy G, Mazumder UK, Gupta M. Antipyretic potential of *Galega pupurea* root. Int Res J Pharm 2011; 2(11): 151-152.
17. Venkatesh P, Dinakar A, Senthilkumar N. Evaluation of Analgesic and Antipyretic activity of alcoholic extracts of *Boerhaavia diffusa* and *Anisochilus carnosus*. J Current Pharma Res 2012; 10(1): 15-19.

18. Timothy SY, Mava Y, Maspalma ID, Gelu GP, Bulus YD. Evaluation of phytochemistry and antipyretic activities of aqueous and ethanolic leaf extract of Azadirachta indica (Neem). Indo-Glo Res J Pharm Sci, 2011; 1(2): 57-61.
19. Jawale PP, Nirmal SA, Atpade SS, Pal SC and Chavan MJ. Studies on Achras sapota bark. Hamdard Med, 2007; 50: 137-139.
20. Cheng L, Ming-liang H, Lars B. Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial. Acta Pharmacologica Sinica, 2005; 26 (8): 926-933.
21. Gupta MB, Nath R, Srivastava N, Shankar K, Kishor K, Bhargava KP. Anti-inflammatory and antipyretic activities of β-sitosterol. Int J Immunopharmacol, 1996; 18: 693-700.
22. Wagner H, Amason JT, Mata R, Romeo JT. Immunostimulants and adaptogens from plants. Phytochemistry of Medicinal Plants; Plenum Press, New York; 1995: 1-18.
23. Kinsella JE, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acid and eicosanoids, potential effects on the modulation of inflammatory and immuned cells: an overview. Nutrition, 1990; 6: 24-44.