Journal of Ayurvedic and Herbal Medicine 2021; 7(3): 176-181

Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2021; 7(3): 176-181
Received: 12-12-2020
Accepted: 30-06-2021
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www.ayurvedjournal.com
DOI: 10.31254/jahm.2021.7302

Phytochemical Screening and Analgesic activity study of different solvent fractions of Aerial parts of *Operculina turpethum* (L.)

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ABSTRACT

Preliminary phytochemical analysis of ethanol extract of the aerial parts of *Operculina turpethum* L. (Family: Convolvulaceae) exhibited the presence of reducing sugar, phenolic compounds, tannins, flavonoids, carbohydrate, glycosides, alkaloids, acidic compounds, steroids, saponin, and terpenoids. The ethanolic crude extract showed significant ($p<0.0001$) peripheral analgesic activity at the doses of 250 mg/kg (26.47% writhing inhibition) and 500 mg/kg (50.74% writhing inhibition) determined by acetic acid induced writhing reflex in mice as compared to control Diclofenac sodium (77.94%). After solvent fractionation, *n*-Hexane, ethyl acetate, butanol and water fraction of *Operculina turpethum* showed 58.82%, 39.70%, 30.14%, and 20.58% writhing inhibition respectively at the dose of 500 mg/kg body weight. Different phytochemicals present in the extract might be responsible for biological activities found with this study. This research could form the basis of further investigation including pure compounds isolation.

Keywords: *Operculina turpethum* L., Phytochemical study, Analgesic activity.

INTRODUCTION

Operculina turpethum (L.) is a large perennial herb (Family: Convolvulaceae), hairy vines growing 4 to 5 meter in length. The leaves are alternate, very variable in shape, ovate, oblong and truncate or cordate at the base. The flowers are large, axillary and solitary. Fruit is a capsule with conspicuous enlarged sepals and thickened pedicles. It is actually not a purgative but a mild laxative. *Operculina turpethum* is also known as Turpeth or Indian Jalap and it is widely distributed throughout India and sometimes cultivated as an ornament in gardens. In many countries, the plant is used as traditional medicine. The root is used to relief pain in scorpion sting and snake bite. The roots are bitter, acrid, sweet, thermogenic, purgative, carminative, antihelmintic, expectorant, antipyretic, hepatic, stimulant and hydragogue. They are useful in colic constipation, dropsy, vitiated conditions of vata, paralysis, myalgia, arthralgia, pectoralgia, bronchitis, obesity, helminthiasis, gastropathy, ascites, inflammations, intermittent fever, leucoderma, puritus, ulcers, erysepelas, haemorrhoids, tumors, jaundice, ophthalmia, employed in drug formulations, dropsical effusions and rheumatism [1, 2].

The plant aerial parts contain turpethosides A, B glycosidal resin and acid glycosides turpethic acids A-C. Four new dammarane-type saponins separated from plant roots are operculinosides A, B, C, D [3]. The various new triterpenoids and steroidal esters were separated from root methanolic extract of plant are 3α,7α-epoxy lanost-5,25-dien-3β-ol, lanost-5,25-dien3α-ol, 4β-hydroxy-3α, 7α-epoxy stigmast-(Z)-5, 22-dien3β-tetradecanoate, 3α,7α-epoxy stigmast-5, 20-dien-3β- hexadecanoate, 12β-hydroxy-3α, 7α-epoxy lanost-(Z)- 5, 20,22-trien-26-oic acid-3β-tetradecanoate and 3α, 7α- epoxy stigmast-(Z)-5,20,22-trien-28-oic acid-3β- hexadecanoate [4]. The Stigma 5, 22-dien 3 O-β-D-Glucopyranoside obtained from alcoholic extract from the roots of the plant [5].

The roots of the plant contain various bioactive compounds such as β-sitosterol, Scopoletin, Betulin, Cycloartenol, Lanosta-5-ene, Coumarin, acrylamide 3- (4-hydroxy-phenyl) -N-[2-(4-hydroxy-phenyl)-ethyl] and salicylic acid [6].

So, as part of our ongoing research, here we evaluated phytochemical groups present in this plant. Analgesic activity of crude ethanolic extract and different fractions of *O. turpethum* were also investigated.

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MATERIAL AD METHODS

Chemicals and Reagents

Ethanol, Mercuric iodide, Potassium iodide, Bismuth nitrate, Tartaric acid, Copper sulphate, Ninhydrin, Sulfuric acid, Sodium potassium Tartarate, *α*-naphthol, Distilled water, Nitric acid, Sodium bicarbonate, Sodium hydroxide, *n*-Hexane, Ethyl acetate, Butanol, Acetic acid, Methanol, Gallic acid, Ferric chloride, Folin-Ciocalteu's reagent, Hydrochloric acid were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All chemicals and reagents were of analytical grade and of high purity.

Reference drugs

Diclofenac Sodium was collected from Beximco Pharmaceuticals Ltd., Bangladesh.

Experimental animals

Swiss-Albino mice of both sex (20-25 gm body weight) were purchased from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) animal house. The animals were provided with standard diets (ICDDR, B formulated) and kept in the standard polypropylene cages and tap water. The animals were allowed to acclimate for 7 days before starting the experiments in the animal house of the Pharmacy Discipline, Khulna University, Bangladesh under standard Laboratory conditions (relative humidity 55-60%, room temperature 25±2^0 C and 12 hours light: dark cycle). All experiments using mice were carried on an isolated and noiseless condition and following the guidelines of the Animal Ethics Committee.

Plant collection and extraction

The aerial part of *O. turpethum* L. was collected from the Khulna University campus, Khulna, Bangladesh. The time of collection was January 19, 2018 at the daytime. During collection, any type of adulteration was strictly prohibited. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen was submitted (voucher specimen no. 45950 DACB**)** for future reference.

The plant was dried by shade drying to ensure the active constituents free from decomposition. After finishing drying, the sample was made coarse powder using mechanical grinder and the powder was kept in a suitable container. About 200 g of powder was extracted by maceration over 15 days with 1000 mL 96% ethanol accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. Then concentrated extract was taken in beaker, the opening of beaker was wrapped by a sheet of aluminum foil to which perforation was done for evaporation of ethanol and was kept in dry and cool place for some days and at last evaporation was done under fan until dried. It rendered concentrate of greenish black color paste type [7]. The extract thus obtained was used for experimental purposes.

Fractionation

At first 2 gm of ethanolic extract of *O. turpethum* was dissolved in 100mL distilled water and sonicated to prepare a homogeneous solution. Then the solution was transferred to a separating funnel. 100 mL of *n*-hexane solvent was added to the funnel. The two solutions were mixed together vigorously. The funnel was then inverted and the tap was carefully opened to release excess vapor pressure. The separating funnel was set aside to allow for the complete separation of the phases. The non-polar compounds are more soluble in *n*-hexane than in water. So, they would be dissolved in *n*-hexane. The top and the bottom tap are then opened and the two phases are released by gravitation. This process was repeated three (03) times to get proper separation. After partitioning with *n*-hexane, same procedures were applied with ethyl acetate and butanol, to get ethyl acetate and butanol fractions respectively. Then the remaining fraction was water fraction [8].

Phytochemical screening

The Phytochemical screening of crude extract of *O. turpethum* was performed by the standard methods [9, 10].

Test for alkaloids

a) Mayer's Test: Test solution (1 mL) was taken in a test tube and some drops of Mayer's reagent (Potassium mercuric iodide solution) were added into it and cream color precipitate was observed.

b) Dragendroff's Test: Test solution (1 mL) was taken in test tube and some drops of Dragendroff's reagent (Potassium bismuth iodide solution) were added into it and observed for reddish brown precipitate.

Test for tannins

$FeCl_3$ Test: About 0.5 mg of dried powdered samples were boiled in 20 mL water in test tubes and filtered. A few drops of 0.1 % ferric chloride solution was added and observed for brownish green or blue-black coloration.

Test for Steroids

In an aliquot of 2 mL of the extract solution few drops of conc. H_2SO_4 was added by means of a pipette via the side of the test tubes. Formation of reddish brown ring at the interface of the two liquids denotes the presence of steroids. Commercially available norgesterol was used as standard for this.

Test for Terpenoids

Salkowski Test: Test solution (1 mL) was taken in a clean and dried test tube and 2 mL chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

Test for Reducing sugars

Fehling Test: Test sample of 1 mL was taken into a clean and dried test tube and 0.5 mL of Fehling A and Fehling B solutions were added to it, boiled and observed for brick red coloration.

Tests for Combined Reducing Sugar

1 mL of aqueous extract of plant material was boiled with 2 mL of dilute hydrochloric acid for 5 minutes, then cooled and neutralized with sodium hydroxide solution and then Fehling's test was performed as described above. A red or brick red color precipitate formation indicates the presence of a combined reducing sugar.

Test for Saponins

Froth test: Test solution (1 mL) was placed in a test tube containing water and shaken well and noted for a stable froth that persists for at least 2 min.

Test for Glycosides

A small amount of an alcoholic extract was taken in 1 mL of water. A few drops of aqueous NaOH were added. A yellow color indicates the presence of glycosides.

Tests for Phenolic Compounds

Ferric Chloride Test: An aliquot of 2 mL aqueous solution of the extract was taken in a test tube. Then 1 mL of 5% (w/v) aqueous ferric chloride solution was added in the test tube. Dark green or bluish black color precipitate indicates the presence of phenolic hydroxyl groups. Here aqueous solution of gallic acid was used as standard [10].

Lead Acetate Test: An aliquot of 2 mL aqueous solution of the extract was taken in a test tube. Then 1 mL of 10% (w/v) aqueous lead acetate solution was added in the test tube. White color precipitate indicates the presence of phenolic compounds. Here aqueous solution of gallic acid was used as standard [10].

Tests for Tannins

Ferric Chloride Test: 5 mL solution of the extract was taken in a test tube. Then 1 mL of 5% ferric chloride solution was added. Greenish black precipitate indicates the presence of tannins. Rose petal was used as standard for this.

Potassium Dichromate Test: 5 mL solution of the extract was taken in a test tube. Then 1 mL of 10% Potassium dichromate solution was added. A yellow precipitate indicates the presence of tannins. Here rose petal was used as standard.

Test for Flavonoids

2 methods are used for this using quercetin as standard.

i. 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicates the presence of flavonoids. The yellow color disappears on standing.

ii. 0.2 gm extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. Formation of yellow color and disappearance of color indicate the presence of flavonoid.

Test for Gums

5 mL solution of the extract was taken and then Molish's reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums. Liquid gum was used as standard for this [10].

Tests for Proteins & Amino acids

Xanthoprotein Test: Some drops of concentrated nitric acid was added by the sides of the test tube into 1 mL of aqueous extract and then observed for formation of yellow color which indicates the presence of xanthoprotein. Here, egg albumin was used as standard.

Ninhydrin Test: About an aliquot of 0.25% w/v ninhydrin reagent was added into 1 mL aqueous extract solution. Then it was boiled for few minutes. Formation of blue color showed the presence of amino acids [13].

Tests for Acidic Compounds

To the alcoholic extract, sodium bicarbonate solution was added and observed for the production of effervescences. Production of effervescences indicates the presence of acidic compound. Salicylic acid was used for standard in this case [14].

Determination of analgesic activity

The analgesic activity of the crude extract and different fraction of *O. turpethum* was investigated using acetic acid induced writhing model in mice. Experimental animals were chosen randomly and divided into eight groups denoted as Control group, Positive control group and Test group I (Crude 250 mg/kg) and Test group II (Crude 250 mg/kg) consisting of five (05) mice in each group. Another four groups of mice were for different fraction of *O. turpethum* (n-hexane, ethyl acetate, butanol and water fraction). Control group was given orally 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group was given orally diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with crude ethanol extract sample orally at the dose of 250 and 500 gm/kg body weight. Another four groups were treated with test sample orally at the dose of 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then acetic acid solution (0.7%) was given to each group of mice intra-peritoneally to induce writhing. After 5 minutes was kept for absorption of acetic acid and number writhing was counted for 15 minutes. The animals do not always show full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing according to Whittle, *et al.* 1964 and Ahmed *et al.* 2004 [15, 16].

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 5 statistical package (Graph Pad Software, USA).

RESULTS AND DISCUSSION

The preliminary phytochemical analysis exhibited the presence of Reducing sugar, Phenolic compounds, Tannins, Flavonoids, Carbohydrate, Glycosides, Alkaloids, Acidic compounds, Steroids, Saponin and Terpenoids in the extract of *O. turpethum* (Table 1).

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and disease preventive properties (phytochemicals). Discovery of novel drugs depending on the pharmacological as well as pathological properties, the critical information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts.

Phenolic compounds, secondary plant metabolites abundantly found in both edible and non-edible plants possess biological properties of antioxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protection, improvement of the endothelial function, as well as inhibition of oxidative damage of DNA [17], angiogenesis and cell proliferation activity [18]. Tannins have been reported to possess anticarcinogenic and antimutagenic potentials as well as antimicrobial properties. The phytochemicals of flavonoids, polyphenols and tannins, quinones, terpenoids and essential oils and alkaloids show antimicrobial activity [19].

Table 1: Result of chemical group test of crude ethanol extract of *O. terpethum*.

Phytochemical Group	*O. terpethum*
Reducing sugar	+
Combined reducing sugar	+
Phenolic compounds	+
Tannins	+
Flavonoids	+
Saponin	+
Gums	+
Steroids	+
Terpenoids	+
Alkaloids	+
Glycosides	+
Protein & amino acids	-
Acidic compounds	+

The test results exhibited that ethanol extract of *O. turpethum* showed significant inhibition of writhing reflex by 26.47% and 50.7353% respectively at the dose of 250 mg/kg and 500 mg/kg body weight and n-hexane, ethyl acetate, butanol, and water fraction of *O. turpethum* showed 58.82%, 39.706%, 30.147%, and 20.588% writhing inhibition respectively at the dose of 500 mg/kg, while the standard drug Diclofenac Sodium inhibition was found to be 77.94% at a dose of 25 mg/kg body weight (Table 2 & 3, Figure 1 & 2). It was observed that the n-hexane fraction of *O. turpethum* exhibited better dose-dependent response than other three fractions. From the above discussion we can conclude that *O. turpethum* contains some non-polar compounds that are responsible for analgesic activity.

Phytoconstituents like terpenoids, gums, flavonoids and tannins are responsible for analgesic activity which are present in *O. turpethum*. Further investigation may be conducted using pure compounds of this extract which will determine the responsible compound for this pharmacological effect.

Table 2: Data presentation of effect of *O. terpethum* extract on acetic acid induced writhing of mice.

Administered dose to mice group	No. of mice	Weight (gm) of mice	Dose	Writhing of mice	Average Writhing
Negative Control	1	32	0.32	32	27.20
	2	33	0.33	26	
	3	35	0.35	23	
	4	31	0.31	27	
	5	34	0.34	28	
Positive Control Diclofenac Na (25 mg/kg)	1	33	0.33	6	6.0
	2	31	0.31	4	
	3	32	0.32	5	
	4	34	0.34	7	
	5	35	0.35	8	
Crude extract of *O. terpethum* (250 mg/kg)	1	30	0.3	20	20
	2	32	0.32	18	
	3	29	0.29	22	
	4	30	0.3	21	
	5	32	0.32	19	
Crude extract of *O. terpethum* (500 mg/kg)	1	32	0.32	11	13.4
	2	30	0.3	13	
	3	31	0.31	16	
	4	29	0.29	15	
	5	34	0.34	12	
n-hexane fraction of *O. terpethum* (500 mg/kg)	1	32	0.32	11	11.20
	2	33	0.33	14	
	3	35	0.35	12	
	4	31	0.31	10	
	5	33	0.33	9	
Ethyl acetate fraction of *O. terpethum* (500 mg/kg)	1	35	0.35	16	16.40
	2	33	0.33	17	
	3	32	0.32	15	
	4	33	0.33	18	
	5	31	0.31	16	
Butanol fraction of *O. terpethum* (500 mg/kg)	1	24	0.24	20	19.0
	2	29	0.29	17	
	3	32	0.32	18	
	4	30	0.3	21	
	5	28	0.28	19	
Water fraction of *O. terpethum* (500 mg/kg)	1	25	0.25	20	21.60
	2	29	0.29	23	
	3	28	0.28	19	
	4	30	0.3	24	
	5	29	0.29	22	

Table 3: Statistical evaluation of effects of the extracts on acetic acid induced writhing of mice.

Administered dose to mice Group	Mean of writhing	% Writhing	% Inhibition of writhing	SD	SEM	t-test (value of p)
Negative control	27.2	100	-----	3.27	1.46	--------
Positive Control Diclofenac Na (25 mg/kg)	6.0	22.06	77.94	1.58	0.71	13.047 ($p < 0.0001$) Significant
Crude extract of *O. terpethum* (250 mg/kg)	20	73.53	26.47	1.58	0.71	4.4313 ($p < 0.002$) Significant
Crude extract of *O. terpethum* (500 mg/kg)	13.40	49.26	50.74	2.07	0.93	7.9674 ($p < 0.0001$) ignificant
n-hexane fraction of *O. terpethum* (500 mg/kg)	11.20	41.18	58.82	1.92	0.86	9.4281 ($p < 0.0001$) Significant
Ethyl acetate fraction of *O. terpethum* (500 mg/kg)	16.40	60.29	39.71	1.14	0.51	6.9714 ($p < 0.0001$) Significant
Butanol fraction of *O. terpethum* (500 mg/kg)	19.0	69.85	30.15	1.58	0.71	5.0468 ($p < 0.001$) Significant
Water fraction of *O. terpethum* (500 mg/kg)	21.60	79.41	20.59	2.07	0.93	3.2332 ($p < 0.01$) Significant

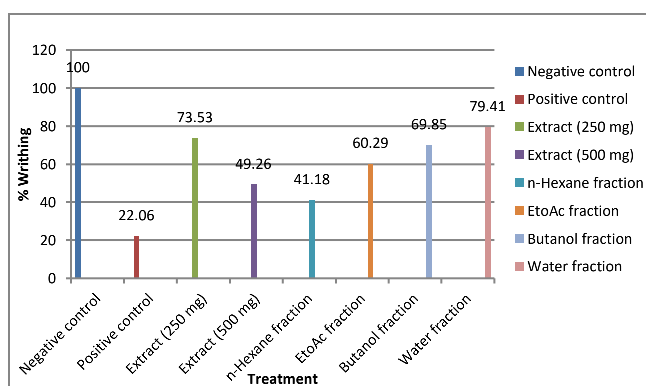

Figure 1: % Writhing vs treatment of standard drug and extract on acetic acid induced writhing in mice.

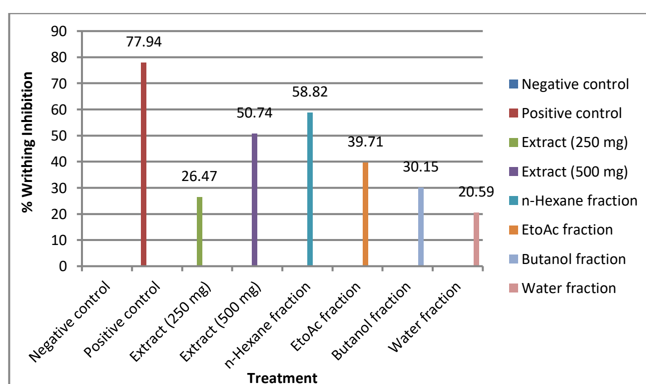

Figure 2: % Writhing inhibition by the standard drug & extract.

CONCLUSION

The extracts of many plants used in traditional medicine contain curative agents that are used in many modern medicines. So, identification of the nature of the compounds present in extracts is essential to evaluate the biological activity of the extract. It can be concluded that ethanol extract of *O. terpethum* is very useful and effective and may be potential source of novel bioactive compounds. For this reason, the extract of the plants should be studied further to isolate and purify the active compounds.

Conflict of Interest

None declared.

Financial support and sponsorship

Nil.

REFERENCES

1. Tasleem Ahmad, Mohd Kashif Husain, Mohd Tariq, Javed Inam Siddiqui, Mohd Khalid, Mohammed Wasim Ahmed, Munawwar Husain Kazmi. A Review on *Operculina turpethum*: A Potent Herb of Unani System of Medicine. *Journal of Pharmacognosy and Phytochemistry,* 2017; 6(1):23-26.
2. Huda Nafees, Sana Nafees and Syed Nizamudeen. A comprehensive review on Turbud *Operculina turpethum* (L): A potential unani drug. *International Journal of Herbal Medicine,* 2020; 8(4):88-92.
3. Ding, W., F. Zeng and L. Xu. Bioactive dammarane type saponins from *Operculina turpethum*. *Journal of Natural Product*, 2011; 74(9):1868-1874.
4. Shuaib, M., M. Ali and K.J. Naquvi. Triterpenoid and steroidal esters from the roots of *Operculina turpethum* (L.). *Journal of Natural Product Plant Resources*, 2013; 3(3):12-19.
5. Sharma, V. and M. Singh. Therapeutic efficacy of isolated stigma-5,22 dien-3-O-b-D-Glucopyranoside and ethanolic root extract of *Operculina turpethum* against N-Nitrosodimethylamine induced hepatopathy in the liver of mice: Ultrastructural and histological evidences. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014; 6(9):226-230.
6. Gupta, S. and A. Ved. *Operculina turpethum* (Linn.) Silva Manso as a medicinal plant species: A review on bioactive components and pharmacological properties. *Pharmacognosy Reviews*, 2017; 11(22):158.
7. Bart H.J., Extraction of natural products from plants–An introduction, *Ind Scale Nat Prod Extr*, 2011, *1–25*.
8. Vastamäki P., Jussila M., Riekkola, M.L., Continuous two-dimensional field-flow fractionation: a novel technique for continuous separation and collection of macromolecules and particles, *Analyst,* 2005; 130(4):427–432.
9. Harbone J.B., Phytochemical methods, pp. *15–29*, (1973), Chapman and Hall Ltd., London.
10. Trease G.E., Evans W.C., Pharmacagnosy, pp. *176 –180,* (1989), Bailliere Tindall, London.

11. Abubakar M.G., Yerima M.B., Zahriya A.G. and Ukwuani A.N., Acute toxicity and antifungal studies of ethanolic leaves, stem and pulp extract of *Tamarindus indica. Res J Pharm Bio Chem Sci*, 2010; 1(4):104–111.
12. Sofowora A., Medicinal plants and traditional medicine in Africa. pp. 64–79, (1982), John Wiley and Sons ltd., New Jursey.
13. Ayoola G., Coker H., Adesegun S., Adepoju-Bello A., Obaweya K., Ezennia E., Atangbayila T., Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria, *Trop J Pharm Res,* 2008; 07:1019–1024.
14. Yadav R., Agarwala M., Phytochemical analysis of some medicinal plants, *J phyt,* 2011; 3(12):10–14.
15. Whittle B.A., The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics, *Br J Pharmacol Chemother*, 1994; 22:246–253.
16. Ahmed F., Das P.K., Islam M.A., Rahman K.M., Rahman M.M., Selim M.S.T., Antibacterial activity of *Cordyline terminalis* Kunth. Leaves*, J Med Sci*, 2003; 3(6):418–422.
17. Ksouri R., Fallah H., Megdchi W., Trabelsi N., Mhamdi B., Chaieb K., Bakrouf A., Magny C., Abdelly C., Antioxidant and antimicrobial activities of edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents, *Food Chem Toxicol,* 2009; 47:2083–2091.
18. Han D.H., Faubion J., Rooney M., Sorghum L.W., Phenolic acids, their high performance liquid chromatography separation, and their relation to fungal resistance, *J Cereal Sci*, 2007; 60:255–261.
19. Kahkonen M.P., Hopia A.I., Vuorela J.H., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M., Antioxidant activity of plant extracts containing phenolic compounds, *J Agric Food Chem,* 1999; 47:3954–3962.

HOW TO CITE THIS ARTICLE
Karmakar UK, Akter S, Sheikh S. Phytochemical Screening and Analgesic activity study of different solvent fractions of Aerial parts of *Operculina turpethum* (L.). J Ayu Herb Med 2021;7(3):176-181. DOI: 10.31254/jahm.2021.7302

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