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RESEARCH ARTICLE

DETERMINATION OF TOTAL PHENOLICAND FLAVONOID CONTENT AND EVALUATION OF ANTIOXIDANT ACTIVITIES OF *CUSCUTA REFLEXA*

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Abstract

Background: The aim of the study was to identify functional groups and antioxidant activity of the *Cuscuta reflexa* (*C. reflexa*) stems extract. Due to the reach of medicinal components, it has been using among Indian subcontinent people to treat various chronic diseases for ancient era. However, there are lacks of study on antioxidant activities of *C. reflexa* with its potential therapeutic compounds, therefore this study motivated to investigate more on its stems extract for further explore.
Methods: Soxhlet extraction method was employed to extract phenolic compounds from *C. reflexa* stems. The effect of extraction time (1-4 h) and concentration of ethanol (45%, 60%, 75% and 90%) on the percentage of yield, total phenolic (TPC) and flavonoid content (TFC) was investigated. The functional groups of phenolic compounds were characterized by using Fourier Transform Infrared Spectrometry (FTIR). DPPH and ABTS•+ radical scavengers were used to evaluate antioxidant activity.
Results: Data showed the highest percentage of yield (10.22±0.14% w/w), TPC (64.11±0.17%, mg GAE/g d.w.) and TFC (41.08±0.34%, mg QE/g d.w.) at 3 h with 75% ethanol. FTIR results revealed the presence of functional groups associated phenolic compounds which are the band at 1260 cm^{-1} illustrations the C–O groups of polyols existence and C–O–C of ester for presence of quercetin which represent the hydroxy flavonoids in the extract. DPPH and ABTS•+ radical scavengers were showed very potent antioxidant activity with IC_{50} 295.12±1.33 and 245.43±0.78 µg/mL.
Conclusion: Phenolic and flavonoids enriched *C. reflexa* extract may play a potential role as a natural nutritional and therapeutic source in Bangladesh.
Keywords: Antioxidant, *Cuscuta reflexa*, flavonoid content, phenolic content.

INTRODUCTION

C. reflexa is one of the well-known parasitic plants which is frequently utilized as a nutrient ingredient in traditional herbal tonics, functional foods and in alcoholic beverages[1-2]. *C. reflexa* belonging to Convolvulaceae family and commonly known as amarbel or dodder or algushi or swarnalatha. It is an enormously found traditional medicinal herb grown in Bangladesh[3]. In addition, this plant has been found most in tropical countries like Malaysia, Thailand, Afghanistan, and Indian subcontinent such as Bangladesh, India, Pakistan, and Nepal[4]. Different sorts of phenolic constituents were found in *C. reflexa* extracts namely glycosides, sterols, terpenes, unsaturated and saturated fatty acids, saturated aliphatic hydrocarbon[5-6]. The stem extract of *C. reflexa* containing phenol (23.49%)[7], kaempherol and quercitin[8]. Furthermore, many biological active compounds were isolated from this plant extract previously like myricetin, quercetin and kaempferol[9], which possess mainly antioxidant and antidiabetic activities. Moreover, some therapeutically active compounds also been reported like coumarin, α-amyrin, astragalin, linoleic acid, palmitic acid, isorhamnetol, oleic acid, luteolin, stearic acid, ß-sitosterol, n-hentriacontane, and sesamin[10]. The 4-vinylphenol isolated from this plant which is using as a flavoring agent[11]. The stem extract of this plant showed potent antibacterial, antioxidant, anti-inflammatory activities[3]. In addition, diuretic, anti-viral and anti-cancer[12], fever, diaphoretic, insanity, demulcent, melancholy, and fits effects also been claimed for traditional use[13]. Besides, several studies were investigated with the extract of *C. reflexa* for antioxidant[14], antitumor[15], anti-epileptic[16], antihyper-tensive[17], anti-arthritic and nephro-protective[18], anti-obesity[19], antispasmodic[20], antibacterial and anti-fungal[21-22], hypoglycemic, hemodynamic, and antiviral effects[23-26].

Traditionally, people in Bangladesh and Nepal are consuming this plant or extract by using hot water decoction for the therapeutical benefits such as jaundice, tumor, skin infections, pain, and edema[27]. The present study was designed to extract from stems of *C. reflexa* using Soxhlet technique with different concentrations of solvent. Further, the concentrated extract was characterized by determine of total phenolic and flavonoids contents. The important functional group was identified using FTIR fingerprinting technique and determine *in vitro* antioxidant activity which leads this study's novelty and important significance to audience.

METHODS

Sample collection

Fresh *C. reflexa* stems (500 g) were obtained from Dhaka district, Bangladesh in 2019. The plant was identified by a taxonomist in Bangladesh National Herbarium, Dhaka, Bangladesh with a voucher specimen (DACB Accession No. 41879)**.** The stems were manually separated and clean with tap water. The plant sample was kept in a laboratory dryer chamber at 25°C until fully dried for one week. It was crushed by using normal a blender, separating uniform size of samples using a fine mesh strainer No. 100 (Sigma-Aldrich, St. Louis, Missouri, 63103, USA) in 0.149 mm. The separated fine sample powder was packed in a sealed bag for further usage.

Chemicals and reagent

Ethanol (99.5% purity) was obtained from Thermo Fisher Scientific (81 Wyman Street Waltham MA, 02454, USA), gallic acid, quercetin, Folin–Ciocalteu reagent, 2, 20-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate anhydrous, and aluminium chloride salt were procured from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Missouri, 63103, USA) and all other reagents and chemicals used in the present study were of analytical grade.

Extraction of plant samples using Soxhlet technique

The *C. reflexa* stem powder sample (20 g) was placed in the Soxhlet extractor. Different concentrations of ethanol (30, 45, 60, 75, 90%) was employed with feed-to-solvent ratio (1:10, 1:15, 1:20, and 1:25 g/mL). The sample mixture was reflux by using heating mantle with different time point as 1, 2, 3 and 4 h[26]. After reaching the pre-determined extraction time, the extraction solution was left at 25°C to cool. The extraction solution was then filtered using filter paper and left in a beaker for some time. The mixture was further concentred to dry using a Buchi R-215 rotary evaporator (BUCHI Malaysia Sdn. Bhd. MY – 47301 Petaling Jaya, Selangor, Malaysia).

Determination of total phenolic content

The Folin-Ciocalteu reagent procedure was employed to determine the TPC of *C. reflexa* stem extract with partial modification of previous method described by Alara *et al*.,[26]. *C. reflexa* stem extract at 5 g/L (1 mL) and 200 µL of Folin-Ciocalteu reagent were mixed at room temperature. After 5 minutes, 0.2mM Na_2CO_3 (0.6 mL) solution was mixed well with the previously prepared mixture (Extract + Folin-Ciocalteu reagent). The absorbance was taken at 560 nm by UV-vis Spectrophotometer (Shimadzu UV-1800, Kyoto 604-8511, Japan). Subsequently, the mixture was kept at 25°C for two hours. TPC concentration of the extract was then measured using the gallic acid standard calibration curve (ranging from 50 to 500 mg/L). TPC was calculated as equivalent to mg GAE/g d.w. using below equation-

$$TPC = \frac{c * V}{m}$$

Where, c=TPC concentration (mg/L), V=volume (L) of solvent used in the extraction, and m=weight (g) of the dried sample used.

Total flavonoid content in the extracts

The quantitative determination of TFC for the extract of *C. Reflexa* was done by using the methods described in the studies of Alara *et al*.,[26]. In brief, an aliquot of 100 µL (1 g/L) plant extract and the same volume of 2% $AlCl_3$ (100 µL) solutions were vigorously mix. Afterwards, mixture solution was permitted to place at 25°C for 60 min. Then supernatant was collected from previous mixture, absorbance measured at 560 nm with UV-vis Spectrophotometer (Shimadzu UV-1800, Kyoto 604-8511, Japan). TFC was calculated using below equation-

$$TFC = \frac{c * V}{m}$$

Where c=TPC concentration (mg/L), V=volume (L) of solvent used in the extraction, and m=weight (g) of the dried sample used.

FTIR analysis

To do FTIR, 1.5–2.0 g of plant sample was gently mixed with 200 mg of solid KBr and ground to make a pellet. The standard device was used to make pellets under vacuum and pressure (75 kN cm^{-2}) for 2–3 minutes (Figure 1). These pellets were then used for spectral analysis of functional groups by FTIR. The spectral resolution was 4 cm^{-1} with a 400–4000 cm^{-1} scanning range[27].

In vitro antioxidant activities of the extract

DPPH assay

Verification has been done on the hydrogen atom or donating ability of electron of extract from *C. reflexa* through DPPH assay[3]. Briefly, 200 µL of 0.1 mM DPPH was added with 200 µL of extract or ascorbic acid, respectively. The mixture was then serially diluted to different concentrations ranging from 100 to 500µg/mL. After incubating the mixture in a dark place for 30 minutes, the absorbance of the sample was measured at 560 nm. The IC_{50} of the sample was calculated through the sample absorbance, where each measurement was done as triplicate. The data was represented as mean±SD. The DPPH radical scavenging activity was determined according to below equation-

$$\%\ DPPH\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where, $A_{control}$= absorbance of solvent and DPPH solution, A_{sample}= absorbance of *C. reflexa* extract and DPPH solution.

ABTS assay

Scavenging ability (ABTS) of *C. reflexa* extract or ascorbic acid was determined by previously described assay[26]. In brief, *C. reflexa* extract (150 µL) was serially diluted to different concentrations ranging from 100 to 500 µg/mL. Then, ABTS solution (285 µL) (2.45 mM potassium persul fate solution and 7mM ABTS) was added into the extract solution. After incubating the mixture in a dark place for 120 minutes, the absorbance of the sample was measured at 734 nm. Afterwards, the IC_{50} of the sample was calculated through the sample absorbance, where each measurement was done as triplicate. The data was represented as mean±SD. The ABTS radical scavenging activity was determined according to below equation-

$$\% \text{ ABTS inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$= absorbance of solvent and ABTS solution, A_{sample}= absorbance of *C. reflexa* extract and ABTS solution.

Statistical analysis

Each experimental test was performed in triplicate. A statistical method analysis of variance (ANOVA, IBM, SPSS 20.0, Chicago, Ill., USA) with $p<0.05$ considered as significance difference.

RESULTS

Soxhlet Extraction

The determination of the recoveries of Soxhlet extraction was correlated with the yields of TPC and TFC. It is depending on the concentration of extraction solvent like ethanol and extraction duration. The extraction time and the concentration of ethanol are determined by the recoveries of extracts, TPC, and TFC from *C. reflexa* using the Soxhlet extraction technique. Duration of extraction is authoritative in minimizing cost and energy of the whole extraction process. Among the key factors, extraction time plays an important role in altering the recovery capacity of phenolic contents from herbal crude extract. The reason behind this is the overexposure of the plant sample on heating degrades the phenolic compounds. That is why, it is very important to fix the exact extraction duration to obtain maximum recovery of the targeted compounds. In this study, the Soxhlet extraction process was fixed with different concentration of ethanol at 1:10 g/mL feed-to-solvent ratio. The extraction time was varied as 1, 2, 3, and 4 hours. After completion of extraction time, the maximum yields of extract (Figure 1A), TPC (Figure 1B), and TFC (Figure 1C) were presented.

In this technique, the maximum yield was observed at 3 h with 75% of ethanol, therefore the TPC and TFC were also showed maximum quantity at the same time. However, 60% and 90% of ethanol extract exhibited almost equal quantity of TPC at 3 h. Interestingly, 45%, 60% and 90% of ethanol extract showed around same quantity of TFC at 3 h. Over all, the yields were declined drastically and it showed almost 0% at 4 h. Therefore, this study found that the % yields of extracts is directly correlate to the % yields of TPC and TFC and process also followed in time dependent manner.

FTIR

FTIR was used to determine the functional groups present in the plant extracts. The functional groups of the compounds were selected from obtained peak number in FTIR chromatograph. The representative absorption peaks value is reported in Figure 2.

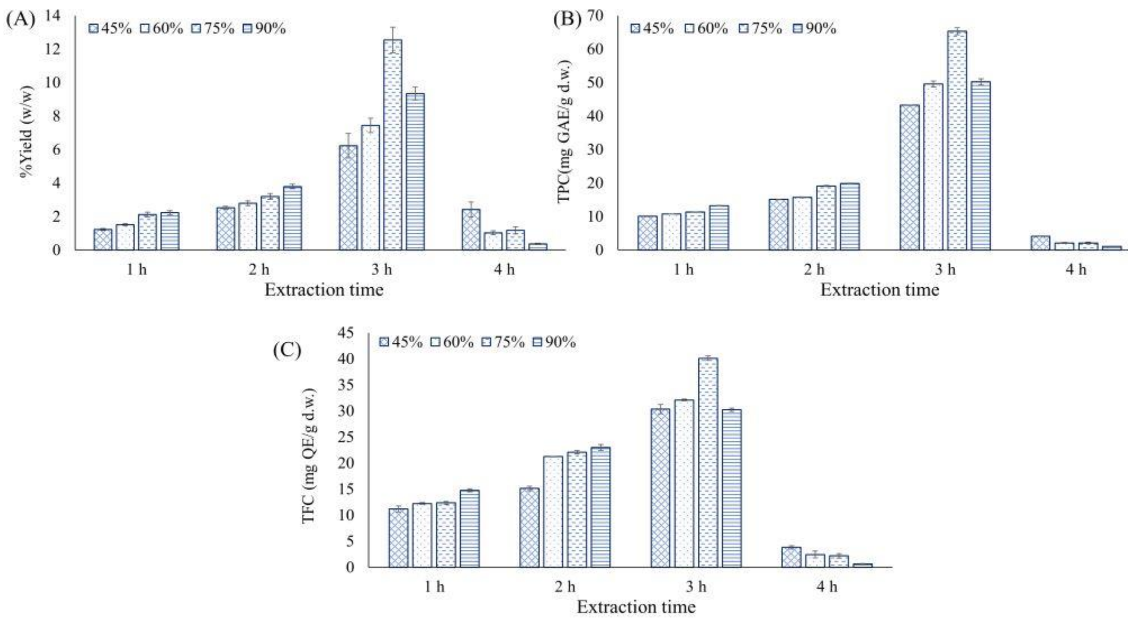

Figure 1: Effects of extraction time and concentration of solvent (ethanol) on the recoveries of extracts % yields (A), TPC (B) and TFC (C) from *C. reflexa* soxhlet extract.

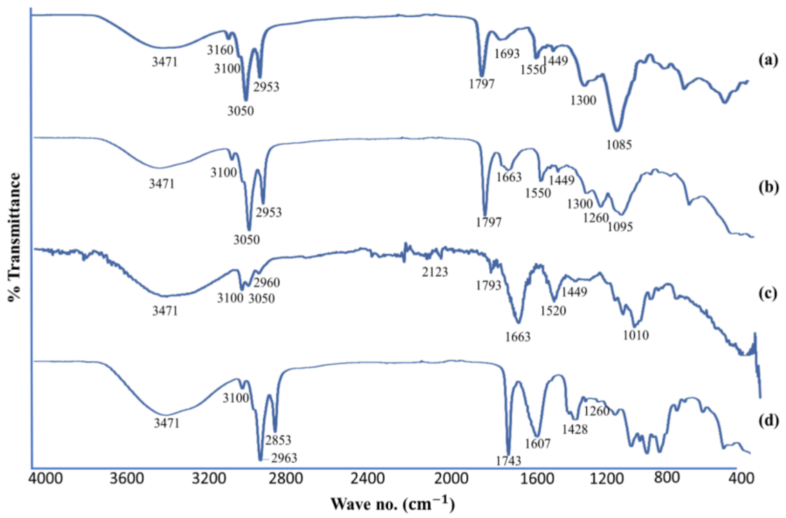

Figure 2: FTIR spectra of *C. reflexa* extract using Soxhlet extraction with (a) 45%, (b) 60%, (c) 75% and (d) 90% ethanol.

The extract represents the phenolic component by the presence of a broad peak at 3471 cm^{-1} with O-H bending. Moreover, few peaks were found that can be attributed to the existence of lipid-carbohydrate in the sample due to the presence of lipids usually assigned in the peak area at 3000-2000 cm^{-1} region and 1500-1200 cm^{-1} represent for carbohydrate. In the extract, the peaks at 2123, 2963, 2960 and 2953 cm^{-1} specify the existence of lipids and 1449, 1428, and 1300 and 1260 cm^{-1} for carbohydrate. The band at 1260 cm^{-1} illustrations the C–O groups of polyols existence which represents the hydroxy flavonoids in the extract. In addition, the sharp peaks were found at 1085, 1010 and 1043 cm^{-1} that indicate the existence of secondary alcohols or ester groups in tested extract. Aromatic ring vibration could be associated with the peak value at 877 cm^{-1}. Glycosides, flavonoids, and carboxylic acid could be associated due to the existence of C=O stretching and N-H bending that possible peak area at 1663 and 1607 cm^{-1}.

***In vitro* antioxidant activity of the extracts**

DPPH and $ABTS^{\bullet+}$ radical scavengers were used to examine the antioxidant properties of *C. reflexa* extract with maximum extraction operating conditions. The results were compared with standard (Ascorbic Acid) shown in the Table 1. It has been found in several studies that the lowest IC_{50} value indicates the strong antioxidant effect. In this sense, the extract showed potent antioxidant in $ABTS^{\bullet+}$ assay with IC_{50} at 245.43±0.78 µg/mL compared to DPPH assay which showed IC_{50} at 295.12±1.33 µg/mL. The obtained data indicated the potent antioxidant properties of the extract. Dissimilar scavenging activities were observed between DPPH and $ABTS^{\bullet+}$ assay with the same extract. It might be due to the different assay followed by the different pathways to exhibit the antioxidant activities.

DISCUSSION

There are numerous methods to recover of antioxidants enrichment of bioactive compounds from natural sources, i.e., cold maceration, Soxhlet extraction, supercritical fluid extraction, microwave assisted extraction, and ultrasound assisted extraction. However, percentage of yield extraction and antioxidant capacity not only vary on the extraction technique but also on the solvent used for extraction. The existence of a variety of antioxidant enrichment bioactive constituents with their polarities and diverse of chemical characteristics may or may not be soluble in a specific solvent[27-28]. Polar solvents are commonly employed for regaining polyphenols from plant materials. Although, methanol, acetone, ethanol, and ethyl acetate containing aqueous mixtures are considering the most suitable solvents for extraction of polyphenols. However, ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption[29-30]. Do *et al.*, studied with 50%, 75% and 100% ethanol Soxhlet extracts, while 100% extracted sample showed the highest TPC at 40.5 mg gallic acid equivalent/g and TFC at 31.11 mg quercetin equivalent/g from *Limnophila aromatica* crude extract[31]. Baba *et al.*, reported the TPC at 45.17±1.70 gallic acid equivalents/g and TFC at 35±2.20 rutin equivalents/g using 100% methanol in Soxhlet extracted up to 4 h[32]. The current study found that the 75% ethanol extract showed the highest amount of TPC (65.32±1.07 gallic acid equivalents/g) and TFC at 3 h. However, the content recovery was drastically reduced at 4 h due to the maximum recoveries was obtained at 2 h and 3 h. The similar finding was reported by Tanruean *et al.*, with 65.45 mg GAE/g extract of TPC in acetone extract of *C. reflexa*[33]. The possibility of bioactive compounds reacts with extracting solvent expanded with increase amount of extraction solvent, leading to higher rates of contents[34]. However, the percentage of yields of antioxidant rich active bioactive compounds will not continue to increase once equilibrium is reached. The solid-to-solvent ratio could considerably affect the equilibrium constant and considered the correlation between yield and solvent use as a steep exponential increase followed by a steady state to give the maximum yield[35]. Moreover, 75% ethanol extract with the highest antioxidant activity was observed by Turkmen *et al.*[36], which is in line with the current findings. Patle *et al.*[37], reported the band obtained at 1520-1500 cm^{-1}, 1449-1400 cm^{-1} and 1260-1200 cm^{-1} due to be NO_2 bending vibration, C–O, and C–O–C of ester for presence of quercetin in the sample extract[37]. Moreover, CH_2 asymmetric and symmetric stretching group was found at 2963 and 2853 cm^{-1} in the sample indicated ethanol[37]. FTIR data revealed the characteristic of the fingerprints of *C. reflexa* extract using the Soxhlet extraction process thatis reflected the existence of various functional groups related to flavonoids or polyphenols compounds.

Limitations of the study: This study completed FTIR finger printing to determine functional groups, TPC, TFC and their antioxidant activities. Due to some limitation, present research project was not able to cover some biological assays such as antibacterial, anti-fungal to increase the depth of this research. Therefore, this study recommends to do further study for more on biological assays.

CONCLUSION

The study found that the time of extraction and solvent concentration had major role in recoveries of extract, TPC and TFC in Soxhlet extraction process. *C. reflexa* extract exhibited potent scavenging activities compared to ascorbic acid, suggested its possible potential use as the natural antioxidant.

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AUTHOR'S CONTRIBUTIONS

A **Azad AK:** conception and design of the work, acquisition, analysis, interpretation of data, the creation of new software used in the work; provided funding acquisition, project administration, and resources **Mohamed F:** wrote the paper, review, editing. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Anjum F, Bukhari SA, Shahid M, Anwar S, Afzal M, Akhter N. Comparative evaluation of antioxidant potential of parasitic plant collected from different hosts. J Food Process Technol 2013; 4(228): 2. *https://doi:10.4172/2157-7110.1000228*
2. Tanruean K, Kaewnarin K, Suwannarach N, Lumyong S (2017) Comparative evaluation of phytochemicals, and antidiabetic and antioxidant activities of *Cuscuta reflexa* grown on different hosts in northern Thailand. Nat Prod Commun 2017; 12(1): 1934578X1701200114. *https://doi.org/10.1177/1934578X1701200114*
3. Azad AK, Laboni FR, Rashid H, Ferdous S, Rashid SS, Kamal N, Islam Sarker Z. *In vitro* evaluation of *Cuscuta reflexa* Roxb. for thrombolytic, antioxidant, membrane stabilizing and antimicrobial activities. Nat Prod Res 2018; 1-4. *https://doi.org/10.1080/14786419.2018.1538216*
4. Pandit S, Chauhan NS, Dixit V.K. Effect of *Cuscuta reflexa* Roxb on androgen-induced alopecia. J Cosmet Dermatol 2008; 7: 199–204. *https://doi.org/10.1111/j.1473-2165.2008.00389.x*
5. Patel S, Sharma V, Chauhan NS, Dixit VK. An updated review on the parasitic herb of *Cuscuta reflexa* Roxb. J Chin Integr Med 2012; 10: 249-255. *https://doi.org/10.3736/jcim20120302*
6. Perveen S, Bukhari IH, Ain QU, Kousar S, Rehman J. Antimicrobial, antioxidant and minerals evaluation of *Cuscuta europea* and *Cuscuta reflexa* collected from difference hosts and exploring their role as functional attribute. Int Res J Pharm Appl Sci 2013; 3: 43-49.
7. Rath D, Panigrahi SK, Kar DM, Maharana L. Identification of bioactive constituents from different fractions of stems of Cuscuta reflexa Roxb. using GC-MS. Nat Prod Res 2018; 32(16): 1977-1981. *https://doi.org/10.1080/14786419.2017.1356837*
8. Nooreen Z, Tandon S, Yadav NP, Ahmad A. (2019) New chemical constituent from the stem of *Cuscuta reflexa* Roxb. and its biological activities. Nat Prod Res 2019; 1-4. *https://doi.org/10.1080/14786419.2019.1669033*
9. Hajimehdipoor H, Kondori BM, Amin GR, Adib N, Rastegar H, Shekarchi M. Development of a validated HPLC method for the stimultaneous determination of flavonoids in Cuscuta chinensis Lam. by ultra-violet detection. DARU J Pharm Sci 2012; 20: 1-6. *https://doi.org/10.1186/2008-2231-20-57*
10. Chatterjee D, Sahu RK, Jha AK, Dwivedi J. Evaluation of antitumor activity of *Cuscuta reflexa* Roxb (Cuscutaceae) against *Ehrlich ascites* carcinoma in Swiss albino mice. Trop J Pharm Res 2011; 10(4): 447-454. *https://doi.org/10.4314/tjpr.v10i4.10*
11. Borole SP, Oswal RJ, Antre RV, Kshirsagar SS, Bagul YR. Evaluation of anti-epileptic activity of *Cuscuta reflexa* Roxb. Res J Pharm Biol Chem Sci 2011; 2(1): 657-663.
12. Jadhav GB, Bhure RR, Mundlod KN, Pingle AP. Effect of *Cuscuta reflexa* extract on experimentally induced hypertension in normal and streptozotocin induced diabetes in rats. Res J Pharm Tech 2020; 13(3): 1351-1355. *https://doi.org/10.1016/j.jep.2016.09.026*
13. Alamgeer Niazi SG, Uttra AM, Qaiser MN, Ahsan H. Appraisal of anti-arthritic and nephroprotective potential of *Cuscuta reflexa*. Pharm Biol 2017; 55(1): 792-798. *https://doi.org/10.1080/13880209.2017.1280513*
14. Kaur A, Behl T, Makkar R, Goyal A. Effect of ethanolic extract of *Cuscuta reflexa* on high fat diet-induced obesity in Wistar rats. Obes Med 2019; 14: 100082. *https://doi.org/10.1016/j.obmed.2019.02.001*
15. Gharib NM, Anvari E, Badavi M. Spasmolytic effect of *Cuscuta pentagona* fruit aqueous extract on rat ileum. Sci J Kurdistan Univ Med Sci 2007; 12(2): 0-0.
16. Kalita D, Saikia J. Ethonomedicinal, antibacterial and antifungal potentiality of *Centella asiatica*, *Nerium indicum* and *Cuscuta reflexa*-widely used in Tiwa tribe of Morigaon district of Assam, India. Int J Phytomedicine 2012; 4: 380-5.
17. Islam R, Rahman MS, Rahman SM. GC-MS analysis and antibacterial activity of *Cuscuta reflexa* against bacterial pathogens. Asian Pac J Trop Dis 2015; 5(5): 399-403. *https://doi.org/10.1016/S2222-1808(14)60804-5*
18. Bernini R, Mincione E, Barontini M, Provenzano G, Setti L. Obtaining 4-vinylphenols by decarboxylation of natural 4-hydroxycinnamic acids under microwave irradiation. Tetrahedron 2007; 63: 9663-9667.

https://doi.org/10.1016/j.tet.2007.07.035

19. Riaz M, Bilal A, Ali MS, Fatima I, Faisal A, Sherkheli MA, Asghar A. Natural products from *Cuscuta reflexa* Roxb. with antiproliferation activities in HCT116 colorectal cell lines. Nat Prod Res 31:583-587. *https://doi.org/10.1080/14786419.2016.1198349*
20. Gupta M, Mazumder UK, Pal DK, Bhattacharya S. Anti-steroidogenic activity of methanolic extract of *Cuscuta reflexa* Roxb. stem and *Corchorus olitorius* Linn. seed in mouse ovary. Indian J Exp Biol 2003; 41: 641–644.
21. Amol P, Vikas P, Kundan C, Vijay P, Rajesh C. *In vitro* free radicals scavenging activity of stems of *Cuscuta reflexa*. J Pharm Res 2009; 2: 58-61.
22. Rahmatullah M, Sultan S, Toma T, *et al*. Effect of *Cuscuta reflexa* stem and *Calotropis procera* leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. Afr J Tradit Complement Altern Med 2010; 7: 109–112. *https://doi.org/10.4314/ajtcam.v7i2.50864*
23. Pal DK, Mandal M, Kumar S, Padhiari A. Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. Fitoterapia 2006; 77: 589–591. *https://doi.org/10.1016/j.fitote.2006.06.015*
24. Anis E, Anis I, Ahmed S, *et al*. α-glucosidase inhibitory constituents from *Cuscuta reflexa*. Chem Pharm Bull 2002; 50: 112–114. *https://doi.org/10.1248/cpb.50.112*
25. Siwakoti M, Siwakoti S. Ethnomedicinal uses of plants among the Satar tribe of Nepal. J Econ Taxon Bot 2000; 24: 323–333.
26. Alara OR, Abdurahman NH, Ukaegbu CI. (2018) Soxhlet extraction of phenolic compounds from *Vernonia cinerea* leaves and its antioxidant activity. J Appl Res Med Aroma 2018; 11:12-17. *https://doi.org/10.1016/j.jarmap.2018.07.003*
27. Younis U, Rahi AA, Danish S, *et al*. Fourier Transform Infrared Spectroscopy vibrational bands study of *Spinacia oleracea* and *Trigonella corniculata* under biochar amendment in naturally contaminated soil. PLoS One 2021; 16: e0253390. *https://doi.org/10.1371/journal.pone.0253390*
28. Wang H, Helliwell K. Determination of flavonols in green and black tea leaves and green tea infusions by high performance liquid chromatography. Food Res Int 2001; 34:223e7. *https://doi.org/10.1016/S0963-9969(00)00156-3*
29. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15:7313e52. *https://doi.org/10.3390/molecules15107313*
30. Bonoli M, Verardo V, Marconi E. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. J Agric Food Chem 2004; 52:5195e200. *https://doi.org/10.1021/jf040075c*
31. Do QD, Angkawijaya AE, Tran-Nguyen PL, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014; 22(3): 296-302. *https://doi.org/10.1016/j.jfda.2013.11.001*
32. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci 2015; 9(4): 449-454. *https://doi.org/10.1016/j.jtusci.2014.11.001*
33. Tanruean K, Poolprasert P, Kumla J, Suwannarach N, Lumyong S. Bioactive compounds content and their biological properties of acetone extract of *Cuscuta reflexa* Roxb grown on various host plants. Nat Prod Res 2019; 33(4): 544-547. *https://doi.org/10.1080/14786419.2017.1392955*
34. Zhang SQ, Bi HM, Liu CJ. Extraction of bio-active components from Rhodiola sachalinensis under ultrahigh hydrostatic pressure. Sep Purif Technol 2007; 57(2): 277-282. *https://doi.org/10.1016/j.seppur.2007.04.022*
35. Hamdan S, Daood HG, Toth-Markus M, Illés V. Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. J Supercrit Fluids 2008; 44(1): 25-30. *https://doi.org/10.1016/j.supflu.2007.08.009*
36. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin Ciocalteu methods. Food Chem 2006; 99:835e41. *https://doi.org/10.1016/j.foodchem.2005.08.034*
37. Patle TK, Shrivas K, Kurrey R, *et al*. Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV–vis and FTIR spectroscopy. Spectrochim Acta A 2020; 118717. *https://doi.org/10.1016/j.saa.2020.118717*