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Evaluation of *Chenopodium Album* Extracts as Antimalarial Agents Using MTT Assay

Zahida Parveen[1]*, Asma Waheed Qureshi[1,2]*, Sadaf Ali[1], Saira Farman[1], Beenish Khurshid[1], Nosheen Faiz[3]

[1] Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan

[2] Department of Zoology, GC Women University, Sialkot, Pakistan

[3] Department of Statistics, Abdul Wali Khan University, Mardan, Pakistan

* Corresponding authors: zahida@awkum.edu.pk, asma.qureshi@gcwus.edu.pk

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Abstract: This article describes testing the antiplasmodial activity of Chenopodium album plant extracts against P. falciparum isolates using a tetrazolium (MTT) colorimetric assay. This idea is based on the need to develop new antimalarial drugs due to the high resistance of malaria parasites to available drugs. Therefore, we tested extracts of *Chenopodium album* against *P. falciparum* isolates for their antiplasmodial activity in the Mardan district. Fresh blood samples from *P. falciparum* infected patients were tested successfully using a tetrazolium (MTT) colorimetric assay for activity of *Chenopodium album* plant extracts in different solvents including n-Hexane, chloroform, acetone, ethanol, methanol and distilled water. 50% inhibitory concentrations (IC50) were noted 9.961, 5.387, 7.82, 7.9, 4.017, 0.524 µg/mL in these solvents. Our results indicate that all extracts tested inhibited the cultured parasites in concentrations ranging from 0.524 to 9.961 µg/mL, of which the aqueous extract being the most active showed excellent antiplasmodial activity (IC50 = 0.524 µg/mL). The results of this study develop and supplement the search for new antimalarial drugs from natural sources and the MTT assay can be used as a diagnostic test. The novelty of the results lies in the significant antiplasmodial activity of C. album extracts against P. falciparum isolates.

Keywords: plant extract, *Chenopodium album*, *P. falciparum.*

将四晓测定评价“晓提取物”作为抗疟剂的作用

摘要：本文介绍了使用四种比色法测试植物提取物对恶性疟原虫分离株的抗疟原虫活性。这一想法是基于由于疟疾寄生虫对现有药物具有高度耐药性而需要开发新的抗疟药物。因此，我们在马尔丹地区测试了藜提取物对恶性疟原虫分离株的抗疟原虫活性。使用四方比色法成功测试了恶性疟原虫感染患者的新鲜血液样本，以检测不同溶剂（包括正己晓、氯效仿、丙酮、乙醇、甲醇和蒸馏水)中。这些溶剂中的50%抑制浓度为9.961、5.387、7.82、7.9、4.017、0.524微克/毫升。我们的结果表明，所有测试的提取物都以0.524至9.微克/毫升的浓度抑制了培养的寄生虫。其中活性最强的水提取物显示出优异的抗疟原虫活性（50%抑制浓度=0.524微克/毫升）。这项研究的结果并补充了从天然来源寻找新的抗疟药物,并且有四

项/份)可以用于诊断测试。结果的新颖性在于藜提取物对恶性疟原虫分离株具有显着的抗疟原虫活性.

关键词：植物提取物、藜、恶性疟原虫.

Introduction

Malaria is an infectious disease due to which about 3,000,000 African and Southeast Asian people pass away per year. A protozoan parasite of the *Plasmodium* family causes malaria, in which *P. falciparum* is very hazardous and causes almost 90% of all deaths from malaria [1]. The two malarial parasites *Plasmodium ovale* and *Plasmodium malariae* are known to be less prevalent, and the infections caused by these parasites are less severe. On the other hand, *Plasmodium knowlesi*, a parasite also known as a parasite of Old World monkeys, causes severe infections in the people of South East Asia [2].

Due to the growing drug resistance of strains of *P. falciparum*, the issue of controlling the disease of malaria has progressively become more challenging. WHO recommended drugs that block the transmission of malaria, particularly in regions of high *P. falciparum* transmission. The use of Primaquine to prevent the spread of malaria in prevalent regions necessitates medical observation because of the hemolytic toxicity of the medicine [3]. Several strains of *P. falciparum* parasite have shown resistance to various drugs and nowadays resistance to chloroquine is very common in East Africa, South America, and South-east Asia. Due to increased international travel, the issue of malaria does not limit only to tropical countries [4]. In 2009, malaria was a major health hazard causing nearly 8 deaths, and this situation is further infuriating by the increasing resistance to almost all antimalarial medicines [3].

A fundamental role has always been played by innovative leads obtained from nature as the basic source of several antimalarial medicines that have been established into effective drugs by semi-synthetic procedures. The most effective antimalarial medicine known for more than three hundred years is quinine derived from the plant *Cinchona succirubra*, which is the finest example of a new lead from nature. Antiplasmodial activity of various other compounds (derived from plants) such as flavonoids, quassinoids, chalcones, and terpenes has also been identified [5, 6]. Another effective antiplasmodial compound (derived from plant) is artemisinin or qinghaosu. Artemisinin was first investigated in China in the 1970s by extraction from the plant *Artemisia annua*, which has been recognized for its therapeutic properties for a period of time [7]. In the past, treatments based on artemisinin were the mainstay of uncomplicated malaria caused by *P. falciparum*. With the appearance of first cases of artemisinin resistance, there is an instant requirement for new combinations to cure malaria caused by *P. falciparum* [8, 9]. In most endemic areas, a histrionic elevation in the prevalence of *P. falciparum* (a malaria parasite showing resistance to frequently used medicine, e.g. Chloroquine) has arisen and is most probably responsible for high malaria-associated mortality. For the treatment of malaria, which is mainly induced by *P. falciparum* and shows resistance to numerous drugs, there is a crucial and persistent need to recognize alternative drugs. Recognizing the fact that many medicines such as artemisinin and quinine etc. are derived from plants having medicinal properties, it is assured that other fresh antimalarial compounds may also be derived from other plants defined in the literature of traditional medicines [10]. Alternative antimalarial drugs are required that are effective against malaria of high resistance and comparatively cheaper [11]. Natural therapeutic plants for antimalarial activity appear to be effective, and at present, significant antiplasmodial properties have been recognized in plants from several areas of the world where malaria is prevalent [12]-[16].

In vitro assays of fresh antimalarial compounds are based on micro tests against *P. falciparum* in animal models and blood cultures as reported by Krettli [17]. However, *in vivo* testing is still needed, which is costly and time consuming even for the purposes of primary examination. Phillipson and O'Neill described the largest published study on valuating plant extracts [4]. Therefore, an HRP2 (histidine-rich protein 2) assay based on drug reactivity is established to test *P. falciparum* fresh isolates in the field. The WHO schizont maturation assay and the isotopic assay are the two traditional (*in vitro* assays) that have been commonly used for more than 2 decades. An innovative ELISA−centered *in vitro* assay (enzyme-linked immunosorbent assay) based on drug reactivity has been developed for *P. falciparum* testing, which overwhelms most of the issues related to *in-vitro* assays. During culturing for 72 hours, this assay quantifies the production of HRP2 (histidine-rich protein 2) and its reticence to antimalarial medicines [18]. Quantification of metabolically active cells for measuring the results of a cytostatic or cytotoxic treatment to inhibit the growth is usually done by using a hemocytometer (a device for counting blood cells) or a particle counter that detects and counts physical particles. These processes are time wasting that take a lot of work not only intrinsically but also needs to

prepare strict suspensions of distinct cells. These types of complications led to the establishment of quick colorimetric tests, such as the MTT test – one enzyme-based procedure defined by Mosmann [19]. MTT is a tetrazolium salt, decreased to a colored product (formazan) by reduction of enzymes that exist only in metabolically vigorous cells.

Keeping in view the medical importance of drug resistance in *Plasmodium falciparum*, the current study is designed for the evaluation of *Chenopodium album*-derived compounds extracted in different solvents for their antimalarial activity through MTT assays.

1. Materials and Methods

1.1. Plant Sample Collection

Fresh samples of *Chenopodium album* were collected from local areas of Mardan. The plant material (leaves) was washed with distilled water to remove the adhering dust and other particles. The plant materials were dried in air for 3-4 days and then powdered mechanically using a commercial electrical stainless steel blender.

1.2. Preparation of Extracts

The dried leaves (5 g) were extracted in n-hexane (50 mL), chloroform (50 mL), acetone (50 mL), ethanol (50 mL), methanol (50 mL), and distilled water (50 mL) using a solvent extraction method and stored at 4°C. Stock solutions of these extracts were prepared in dimethyl sulfoxide (DMSO).

1.3. Blood Sample Collection

The patient skin was disinfected with ethyl alcohol and 1 mL blood sample was collected by puncturing the vein with the help of a disposable syringe and an EDTA tube. With the help of 2% Giemsa solution, thin and thick blood smears were prepared to assess the parasite (*P. falciparum*) density. A whole of 25 mL of CMM (cell medium mixture) at 1.5% hematocrit was obtained by mixing 0.94 mL of collected blood sample with 24.06 mL of complete RPMI 1640 medium containing 10.43 grams of RPMI 1640 powder, 25 mg of gentamicin, 6 grams of HEPES, 0.5% albumin and dH2O to prepare 1 liter of medium. Adding 28 mL of 7.5% NaHCO3 solution before use [18]. Serial twofold dilutions were inoculated in duplicate into typical 96-well microculture plates following a partially automatic microdilution procedure.

1.4. Semi-Automatic Microdilution Assay

In this technique, a stock solution of 10 mg/mL of the extract was first obtained in the solvent DMSO. Serial dilutions of all extracts were prepared in the same manner by dissolving 1 mL of the stock solution in 1 mL DMSO to make a volume of 2 mL in 1:1. 1 mL from the first dilution was dissolved in 1 mL DMSO to make 1:2. Next, 1 mL of the second dilution was further dissolved in 1 mL DMSO to make 1:4. In the same manner 1 mL of the third dilution was dissolved in 1 mL DMSO to make 1:8. Then 1 mL of the fourth dilution was dissolved in 1 mL DMSO to make 1:16. The same process was carried on until seven dilutions of each extract were prepared in 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64. The volume of seventh dilution was 2 mL in 1:64. 1 mL of the seventh dilution was discarded to leave 1 mL dilution like the other six dilutions. In the A1, A2 and A3 wells, only DMSO was inoculated to serve as the control well. Artemisinin was inoculated in A4 to A12 wells. In the B1 to H1 column and B2 to H2 column, serial dilutions of n-Hexane extract were inoculated in a manner that first dilution in the B1 and B2 wells, second dilution in the C1 and C2 wells and seventh dilution in the H1 and H2 wells were inoculated in duplicate. In the same manner serial dilutions of chloroform extract in the columns B3 to H3 and B4 to H4, serial dilutions of acetone extract in the columns B5 to H5 and B6 to H6, serial dilutions of ethanol extract in the columns B7 to H7 and B8 to H8, serial dilutions of methanol extract in the columns B9 to H9 and B10 to H10, serial dilutions of water extract in the columns B11 to H11 and B12 to H12 were inoculated. The number of the dilutions in the wells was the same (25 µL/well). After this inoculation, the plate was incubated for a whole night at a temperature of 37°C.

1.5. Culture of *Plasmodium*

An equal amount (200 µL) of the CMM (medium+ parasitized blood sample) was added to all wells and incubated at 37°C for 72 hours. These culture plates were then placed in a desiccator containing a burning candle and covered completely. When the candle went off due to limited oxygen supply, the lid of the desiccator was closed completely [20]. After that, the plates in the desiccator were incubated in an incubator at 37°C for 72 hours. After incubation, the culture plates were shifted into a simple freezer set at a temperature of approximately 15°C [18]. The MTT assay was then used to determine *Plasmodium* Cell activity [20]. The main steps of the research process are summarized in Fig. 1.

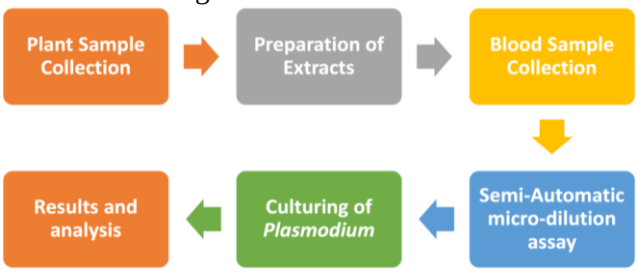

Fig. 1 The main steps of the research process

1.6. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version (GraphPad, La Jolla, CA, USA) 6.01 software. The IC_{50} values (non-linear regression analysis) for the antiplasmodial activity were calculated

from the plots of log inhibitor concentration versus % inhibition. IC_{50} values represent the confidence intervals for the 50% inhibitory concertation parameter.

Seven extracts were prepared from n –hexane to aqueous extracts on the basis of polarity and evaluated for antimalarial activity. Different concentrations of these extracts showed different activities (Fig. 2).

2. Results

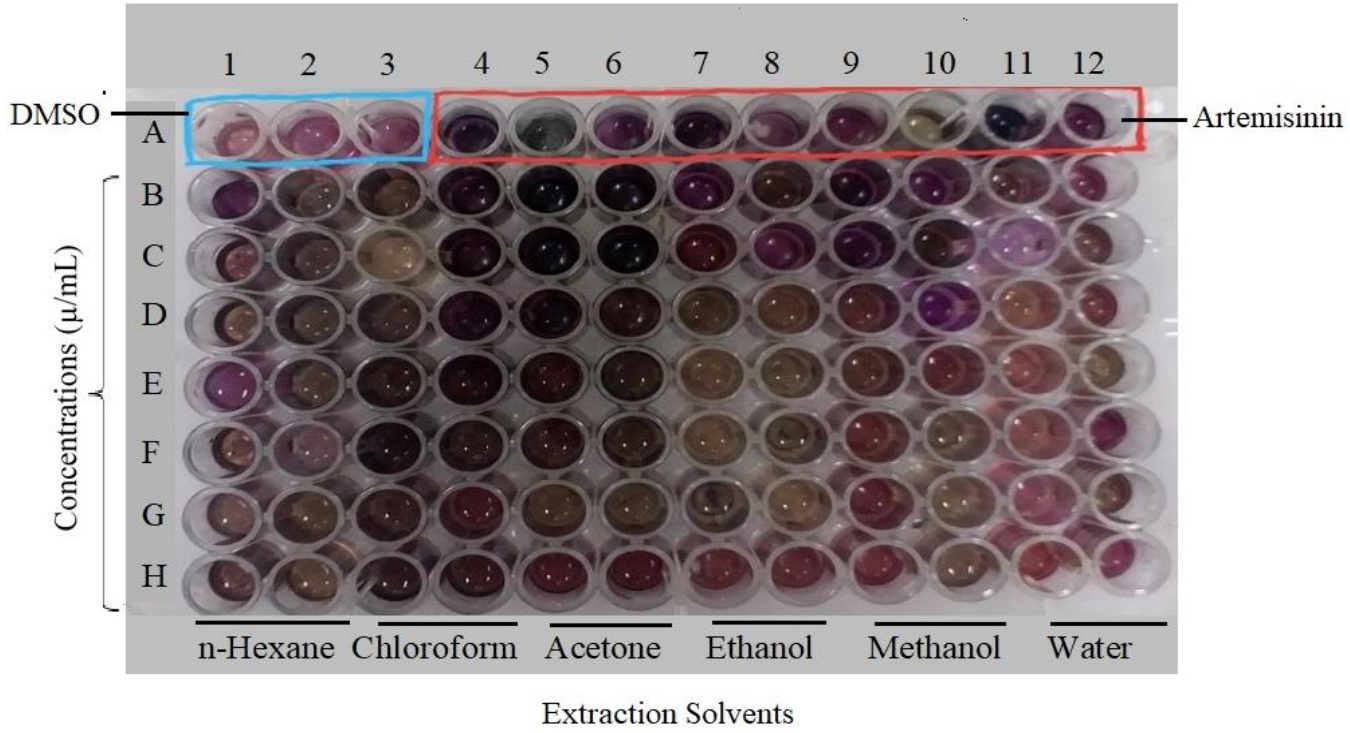

Fig. 2 The ELISA plate (96 wells) after an MTT assay: Wells B1 to H1 contain different conc. of extracts, i.e., 1, 7.5, 15, 31, 62, 125, and 250 µ/mL, respectively in Different solvents i.e., n-Hexane, Chloroform, Acetone, Ethanol, Methanol and Water. Well A1 to A3 serves as control without Drug, A4 to A12 inoculated with Artemisinin.

2.1. The Antiplasmodial Activity of Less Polar Extracts

A concentration range of 1.0 to 250 µg/mL was evaluated for the antiplasmodial activity in less polar solvent extracts including n-hexane, chloroform, and acetone. A dose-response curve showed a trend, which indicates that activity increased with increase in concentration in all three solvents. However, the best comparison was made based on IC50 values which were found to be 9.961 ± 0.1315, 5.387 ± 0.0853 and 7.82 ± 0.3866 for n-hexane, chloroform and acetone respectively (Fig. 3).

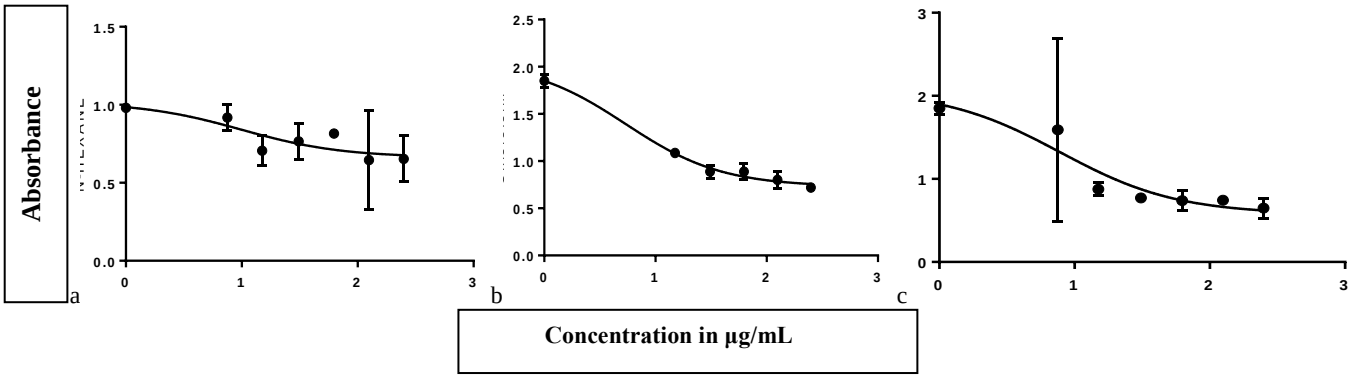

Fig. 3 Antiplasmodial activity of less polar extracts x: (A) Inhibition by n-hexane extract, (B) chloroform extract, and (C) acetone extract. Dose response curve was generated with log inhibitor concentration on X-axis and absorbance on Y-axis.

2.2. The Antiplasmodial Activity of High Polar Extracts

Then high polar solvent extracts including ethanol, methanol and water extracts were evaluated for their antiplasmodial activity. A concentration range of 1.0, 7.5, 15, 31, 62, 125 and 250 µg/mL was evaluated. A dose-response curve was also obtained for high polar extracts by plotting log inhibitor concentration on the X-axis and absorbance on the Y-axis. Where line showed trend, which indicated that activity was increased with increase in concentration and squares showed the data points. After ethanol, the next solvent used was methanol and then distilled water. The best comparison was made on the basis of IC50 values which were found to be 7.9 ± 0.1585, 4.017 ± 0.1119 and 0.524 ± 4.032 for ethanol, methanol and distilled

water respectively. The element tested showed a 50% inhibition of parasite growth in concentrations ranging from 0.5 to 9 µg/mL, and being an active substance revealed IC50 values below 14 µg/mL (see Fig. 4).

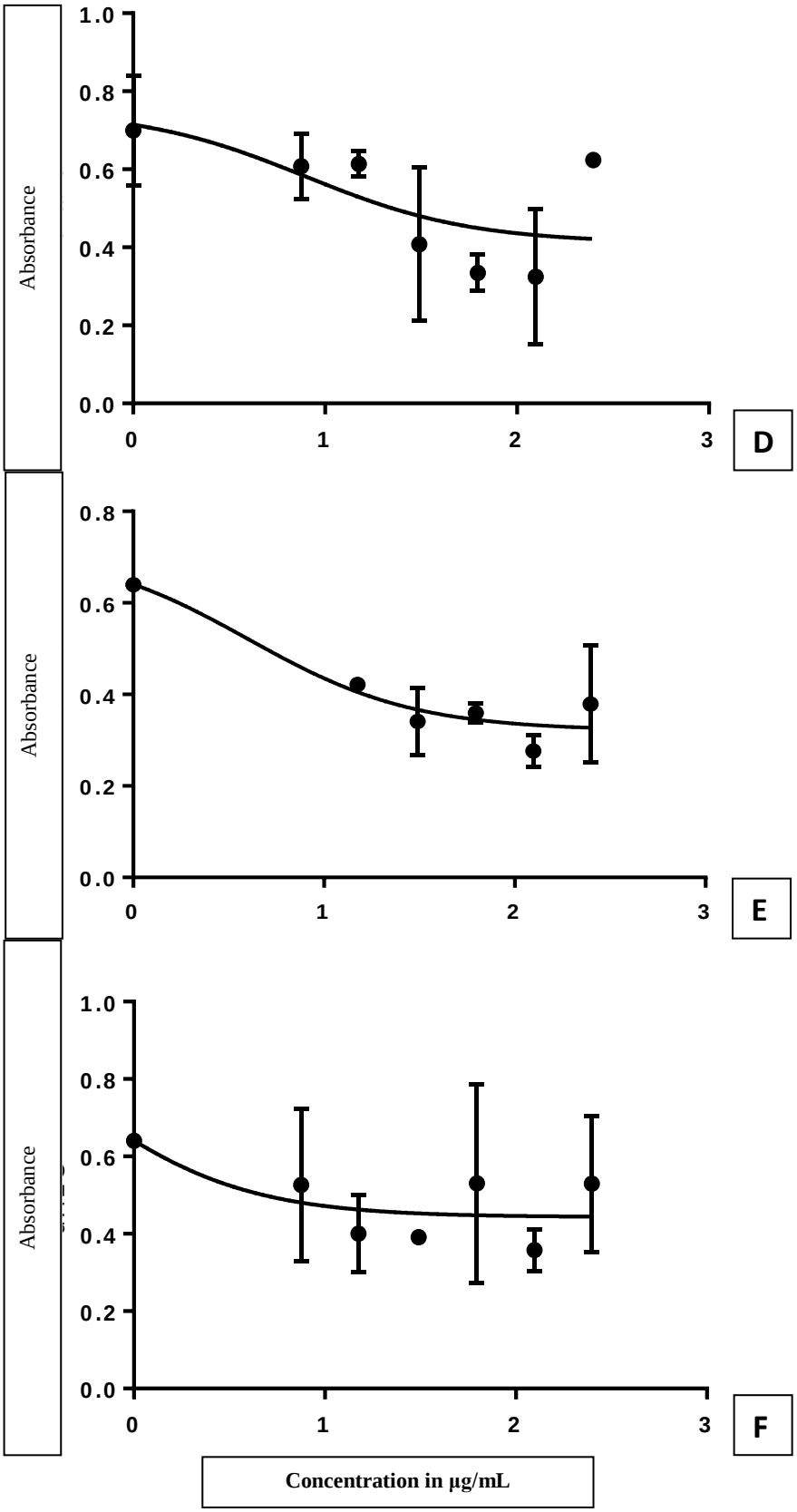

Fig. 4 Antiplasmodial activity of high polar extracts: (D) Inhibition by ethanol extract, (E) methanol extract, and (F) water extract. Dose response curve was generated with log inhibitor concentration on X-axis and absorbance on Y-axis.

The extract prepared in distilled water showed a significant (IC50 < 20 µg/mL) result with IC50 = 0.524 µg/mL, followed by the extract prepared in methanol (IC50 = 4.017 µg/mL), chloroform (IC50 = 5.387 µg/mL), acetone (IC50 = 7.82 µg/mL), ethanol (IC50 = 7.9 µg/mL), and n-Hexane (IC50 = 9.961 µg/mL). The details of the resulting IC50 values are expressed in Table 1.

Table 1 IC50 values (± S.E) values for *C. album* extract against fresh field isolates of *P. falciparum* from District Mardan, Pakistan

Sr. no.	Solvents	IC50 ± S.E in µg/mL
1	n-Hexane	9.961 ± 0.1315
2	Chloroform	5.387 ± 0.0853
3	Acetone	7.82 ± 0.3866
4	Ethanol	7.9 ± 0.1585
5	Methanol	4.017 ± 0.1119
6	Distilled Water	0.524 ± 4.032

3. Discussion

At present time when plasmodial parasites have become resistant to nearly all existing antiplasmodial medicines, avoidance of drug resistance is essential. *In-vitro* tests of drug reactivity are very effective for drug resistance observation. While the preservation of cells and long-standing culture could notably change the pattern of drug resistance of very diverse populations of the parasite, the probability to apply *in vitro* analyzes to fresh isolates of the parasite in the field is critical. However, high parasite densities are required for some traditional tests that prevent the analysis of fresh isolates at parasite densities that occur naturally in diseased patients without any favoritism in sample selection.

The recent rush in the resistance to antimalarial medicines has appeared as a serious hazard to the capacity to cure and prevent malaria in the future. Products obtained from plants in accumulation with other antimalarial compounds chemically synthesized from a natural product have earlier established evidence to produce strong combination remedies against malaria.

The analysis of the leaves of *Chenopodium album* was carried out in the current study. The plant has been found to be rich in protein, lipids, fibers, carbohydrates, vitamins, calcium, phosphorus, zinc, iron, potassium, sodium, magnesium, copper, manganese and nitrogen. Examination of the *C. album* leaves showed that they contain trypsin, simple phenols, polyphenols, tannins, saponins, phytic acid, phytate phosphorus, alkaloids, flavonoids, and oxalates [21, 22].

In Asia, *C. album* is used as a laxative, diuretic, sedative, and also to cure rheumatism [23], as a blood purifier in hepatic disorders, digestive, and in diarrhea for the treatment of dyspepsia, cardiac illnesses, seminal weakness, and intestinal ulcers [24, 25]. The plant was also used conventionally as an anthelmintic against round and hookworms [26] for the treatment of abdominal discomfort, eye disease, throat problems, and cardiovascular diseases [27]. Boiled shoots of the plant are used in constipation [28]. Juice of *C. album* leaves is used for curing burns. The aboveground parts of the plant are used in mixed combination with alcohol for the treatment of arthritis and rheumatism [29].

Though, the most important and active phytoconstituents isolated from different parts of the plant included non-polar lipid, phenols, saponins, lignins, glycosides, flavonoids, alkaloids, ascorbic acid, ferulic acid, p-coumaric acid, caffeic acid, β-carotene, catechin, gallocatechin, xanthotoxin, campesterol, n-triacontanol, stigmasterol, imperatorin, ecdysteroid [26], cinnamic acid amide, crytomeridiol, n-transferuloyl- 4-O-methyl dopamine, β-sitosterol, lupeol and 3 hydroxy nonadecyl henicosanoate [30, 31].

Some scientists have proposed that antimicrobial constituents of plant extracts (alkaloid, terpenoid and phenolic compounds) may cause cell death or inhibit enzymes essential for the biosynthesis of microbe amino acids [32]. Other scientists recognized that the inhibitory effects of these plant extracts allow them to react with proteins of microbial cell membranes and mitochondria, disturbing their structures and altering their permeability [33, 34].

In this study, we confirm that *C. album* has antiplasmodial properties, as revealed by our *in vitro* results. All extracts tested inhibited the cultured parasites in concentrations ranging from 0.524 to 9.961 µg/mL, with the aqueous extract is the most active. This may be due to the high polarity of the water that can dissolve many substances like tannins, saponins, terpenoids and flavonoids. All these compounds provide health benefits. It is waters chemical composition and physical attributes that make it such an excellent solvent and sometimes called the "universal solvent". Our results are in the same line for antiplasmodial against *P. falciparum* isolates. The extract prepared in distilled water showed the most effective result followed by the extract prepared in methanol. Because methanol is also a polar solvent and can dissolve a wide variety of polar compounds like terpenoids, saponins, tannins, polyphenols and flavones. But methanol can dissolve some nonpolar molecules as well. Although chloroform is not very polar and can dissolve a limited variety of compounds like terpenoids and flavonoids, the extract prepared in chloroform also showed good antiplasmodial activity.

Acetone is also a less polar solvent, because of this small polarity acetone mingles only with flavonoids and therefore revealed less significant result than that of the methanol, chloroform and water. Ethanol is a very polar solvent, but the ethyl group in ethanol is nonpolar. Thus, ethanol can dissolve both polar and non-polar substances. Ethanol is known as a good solvent for the polyphenol extraction from plants. In addition, ethanol also dissolves tannins, terpenoids, alkaloids and flavones. The results obtained with ethanol extract showed similarity to the results obtained using extract prepared in acetone. The extract prepared in n-Hexane also showed good results but was less significant than that of all other solvents. Because n-Hexane is very non-polar and can only dissolve non-polar compounds like oils and fats.

These extracts with IC50 values in the small micromolar ranges and vigorous action against *P.*

falciparum parasites justify additional investigation as the compound optimistically to be added to the restricted collection in the brawl against malaria.

An in vitro tetrazolium-based colorimetric assay (MTT assay) was developed for assessing the selectivity of antimalarial drugs. This assay, in contrast to all other assays, measures the potential of drugs to prevent RBCs (red blood cells) destruction by *P. falciparum*.

It has become less common to see the use of the MTT assay in the literature for testing the susceptibility of a disease-causing cell to the action of a chemical agent [35]. This assay is thoroughly being used by the National Cancer Institute of USA for large-scale transmission of potential novel drugs [36]. The MTT test has various benefits, in which the main advantage is its high rapidity due to its ease of use. This assay is comparatively relaxed to arrange and does not require excessive experience. Because it is a partially automatic assay and various well plates are used, this assay assures many compositions and alternatives to be explored without spending extreme resources. Colorimetric assays such as the MTT assay are highly beneficial for the examination of various cell lines and their reaction to several cytotoxic drugs and a combination of drugs.

The challenges for the application of field tests of *in vitro* drug reactivity are extremely elevated. Field analysis requires an extremely sensitive and simple assay that provides a great yield with field isolates and that works within a widespread range of parasite populations. All these requirements are fulfilled by the HRP2-based field assay, which is very sensitive, nonisotopic, partially automated, and extremely simple to perform. The procedure of culturing parasites in the field is very similar to that of the WHO *in vitro* micro-test. However, for automatic reading of the results with an ELISA plate reader, the culture plates may either be transported to a laboratory or may be performed in the field.

As the use of albumin as a protein source is highly advantageous, we used commercial albumin rather than human serum. Another main advantage of commercial albumin is its standardized quality. In addition, the need for handling and preserving serum under field conditions is abolished by the use of albumin. However, the results obtained with human serum and those obtained with commercial albumin may not be the same. The comparison of the results obtained eventually will be easy to relate to the quality and compatible protein contents in the culture medium.

4. Conclusion

4.1. Strength

This assay can calculate antimalarial indices and provide evidence that new compounds are truly effective against malarial parasite. *Chenopodium album* plant extracts in different solvents were shown to be very effective against *P. falciparum* strains.

4.2. Main Findings

Our findings therefore support the use of *C. album* as antimalarial. *In Vivo* evaluation of antiplasmodial active ingredients in solvents of present study is recommended.

4.3. Limitations

The main limitation of the study is small sample size and lack of diversity

4.4. Recommendations, Implications, and Future Research

Further evaluation of these extracts against other *Plasmodium* species should be assessed on a larger level including more samples for diverse areas. This would lead to safer and cheaper drugs against malaria and save millions of lives.

4.5. Academic Contribution to the Existing Literature

This paper is novel because it seeks to contribute to the current debate in the literature for the identification of potential new antimalarial drugs, the MTT-based colorimetric assay was found to be satisfactory. The novelty of the results lies in the significant antiplasmodial activity of C. album extracts against P. falciparum isolates. The use of the MTT assay in the literature for testing the susceptibility of a disease-causing cell to the action of a chemical agent is common [34]. This assay is thoroughly being used by the National Cancer Institute of USA for large-scale transmission of potential novel drugs.

References

[1] DUBAR F., KHALIFE J., BROCARD, J., DIVE, D., and BIOT C. Ferroquine, an Ingenious Antimalarial Drug – Thoughts on the Mechanism of Action. *Molecules*, 2008, 13: 2900-2907. https://doi.org/10.3390/molecules13112900

[2] COX-SINGH J., & SINGH B. Knowlesi malaria: newly emergent and of public health importance. *Trends in Parasitology*, 2008, 24: 406-410. https://doi.org/10.1016/j.pt.2008.06.001

[3] WHO. *World Malaria Report 2010: WHO Global Malaria Programme*. World Health Organisation, Geneva, 2010.

[4] PHILLIPSON J. D., & O'NEILL M. J. Antimalarial and amoebicidal natural products. In: HOSTETTMANN K., & LEA P. J. (eds.) *Biologically Active Natural Products*. Oxford, Clarendon Press, 1987: 49-64.

[5] SAXENA S. P. N, JAIN D. C., and BHAKUNI R. S. Antimalarial agents from plant sources. *Current Science*, 2003, 85(9): 1314-1329. https://www.jstor.org/stable/24108135

[6] KAUR K., JAIN M., KAUR T., and JAIN R. Antimalarials from nature. *Bioorganic and Medicinal Chemistry*, 2009, 17(9): 3229–3256. https://doi.org/10.1016/j.bmc.2009.02.050

[7] BRUCE-CHWATT L. J. Qinghaosu: a new antimalarial. *British Medical Journal*, 1982, 284: 767–768. https://doi.org/10.1136/bmj.284.6318.767
[8] NOEDL H., SE Y., SCHAECHER K., SMITH B. L., SOCHEAT D., and FUKUDA M. M. Evidence of artemisinin-resistant malaria in western Cambodia. *The New England Journal of Medicine*, 2008, 359(24): 2619–2620. https://doi.org/10.1056/nejmc0805011
[9] GANESH D., FUEHRER H.P., STARZENGRÜBER P., SWOBODA P., KHAN W.A., REISMANN J. A. B., MUELLER M. S. K, CHIBA P., and NOEDL. H. Antiplasmodial activity of flavonol quercetin in Bangladesh. *Parasitology Research*, 2012, 110: 2289–2295. https://doi.org/10.1007/s00436-011-2763-z
[10] DHAR R., ZHANG K., TALWAR G. P., GARG S., and KUMAR N. Inhibition of the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite *Plasmodium falciparum* by Neem (*Azadirachta indica*) fractions. *Journal of Ethnopharmacology*, 1998, 61(1): 31–39. https://doi.org/10.1016/s0378-8741(98)00012-9
[11] WERNSDORFER W. H., & TRIGG, P. I. Recent progress of malaria research: chemotherapy. In: WERNSDORFER W. H., & MCGREGOR I. (eds.) *Malaria principles and practice of malariology*. Churchille Livingstone, Edinburgh, 1988: 1569–1674.
[12] PRESBER W., HEGENSCHEID B., HERNANDEZ-ALVAREZ H., HERMANN D., and BRENDEL C. Inhibition of the growth of *Plasmodium falciparum* and *Plasmodium berghei* in vitro by an extract of Cochlospermum angolense (Welw). *Acta Tropica*, 1992, 50: 331–338. https://doi.org/10.1016/0001-706x(92)90068-9
[13] GESSLER M. C., NKUNYA M. H. H., MWASUMBI L. B., HEINRICH M., and TANNER M. Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica*, 1994, 56: 65–77. https://doi.org/10.1016/0001-706x(94)90041-8
[14] FRANSSEN F. F. J., SMEIJSTERS L. J. J. W., BERGER I., and ALDANA, B. E. M. In vivo and in vitro antiplasmodial activities of some plants traditionally used in Guatemala against malaria. *Antimicrobial Agents and Chemotherapy*, 1997, 41: 1500–1503. https://doi.org/10.1128/aac.41.7.1500
[15] MACKINNON S., DURST T., ARNASON J. T., ANGERHOFER C., PEZZUTO J., SANCHEZ-VINDAS P. E., POVEDA L. J., and GBEASSOR M. Antimalarial activity of tropical Meliaceae extracts and gedunin derivatives. *Journal of Natural Products*, 1997, 60: 336–341. https://doi.org/10.1021/np9605394
[16] JENETT S. K., FRANK P. M., ULRICH B., MAHABIR P. G., and ECKART E. In vitro antiplasmodial activity of Central American medicinal plants. *Tropical Medicine and International Health*, 1999, 4(9): 611–615. https://doi.org/10.1046/j.1365-3156.1999.00456.x
[17] KRETTLI A. U. Antimalarial drug discovery: screening of Brazilian medicinal plants and purified compounds. *Expert Opinion on Drug Discovery*, 2009, 4: 95-108. https://doi.org/10.1517/17530050802678127
[18] NOEDL H., ATTLMAYR B., WERNSDORFER W. H., KOLLARITSCH H., and MILLER R. S. A histidine-rich protein 2−based malaria drug sensitivity assay for field use. *The American Journal of Tropical Medicine and Hygiene*, 2004, 6(71): 711–714. https://pubmed.ncbi.nlm.nih.gov/15642959/
[19] MOSMANN T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983, 65: 55-63. https://doi.org/10.1016/0022-1759(83)90303-4
[20] AYISI N. K., OPONG R. A., GYAN B, BUGYEI K., and EKUBAN F. *Plasmodium falciparum*: Assessment of Selectivity of Action of Chloroquine, Alchornea cordifolia, Ficus polita, and Other Drugs by a Tetrazolium-Based Colorimetric Assay. *Malaria Research and Treatment*, 2011: 816250. https://doi.org/10.4061/2011/816250
[21] ADEDAPO A, JIMOH F., and AFOLAYAN A. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of Bidens pilosa and Chenopodium album. *Acta Poloniae Pharmaceutica*, 2011, 68(1): 83-92. https://pubmed.ncbi.nlm.nih.gov/21485705/
[22] SOOD P., MODGIL R., SOOD M., and CHUHAN P. K. Anti-nutrient profile of different Chenopodium cultivars leaves. *Annals Food Science and Technology*, 2012, 13(1): 68-74. https://afst.valahia.ro/wp-content/uploads/2022/09/3-PurvikaSood_2012.pdf
[23] ARORA S. K., ITANKAR P. R., VERMA P. R., BHARNE A. P., and KOKARE D. M. Involvement of NFκB in the antirheumatic potential of *Chenopodium album* L., aerial parts extracts. *Journal of Ethnopharmacology*, 2014, 155(1): 222-229. https://doi.org/10.1016/j.jep.2014.05.026
[24] AGARWAL S. S., YAMREKAR B. P., and PARIDHAVI M. *Clinical useful herbal drug*. Ahuja Publishing House, New Delhi, 2005: 10-12.
[25] KHARE C. P. *Indian medicinal plants*. Springer International Publication, New Delhi, 2007: 141-142.
[26] PRIYA S., YOGESH S., SINGHAI A. K., and ABHISHEK S. Pharmacological and phytochemical profile of *Chenopodium album* linn. *Research Journal of Pharmacy and Technology*, 2010, 3(4): 960-963.
[27] BALDI A., & CHOUDHARY N. K. In vitro antioxidant and hepatoprotective potential of chenopodium album extract. *International Journal of Green Pharmacy*, 2013, 7(1): 50-56. http://www.greenpharmacy.info/index.php/ijgp/article/view/297
[28] GOGOI B., & ZAMAN K. Phytochemical constituents of some medicinal plant species used in recipe during 'Bohag Bihu' in Assam. *Journal of Pharmacognosy and Phytochemistry*, 2013, 2(2): 30-40. https://www.phytojournal.com/vol2Issue2/3.1.html
[29] PAL A., BANERJEE B., BANERJEE T., MASIH M., and PAL K. Hepatoprotective activity of Chenopodium album linn. plant against paracetamol induced hepatic injury in rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013, 3: 55-57. https://innovareacademics.in/journal/ijpps/Vol3Suppl3/2065.pdf
[30] CUTILLO F., DELLA GRECA M., GIONTI M., PREVITERA H., and ZARRELLI A. Phenol and lignans from Chenopodium album. *Phytochemical Analysis*, 2006, 17(5): 344-349. https://doi.org/10.1002/pca.924
[31] JHADE D., PADMAA M. P., and USHA G. Isolation of phytoconstituents from the leaves of Chenopodium album Linn. *Journal of Pharmacy Research*, 2009, 2(7): 1192-1193. https://www.researchgate.net/publication/40765427_Isolation_of_Phytoconstituents_from_the_leaves_of_Chenopodium

_album_Linn
[32] BURT S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods - A Review. *International Journal of Food Microbiology*, 2004, 94: 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
[33] FRIEDMAN M., HENIKA P. R., LEVIN C. E., and MANDRELL R. E. *Antibacterial Activities of Plant Essential Oils and Their Components against Escherichia coli 0157:H7 and Salmonella enterica in Apple Juice*, 2004, 52: 6042–6048. https://doi.org/10.1021/jf0495340
[34] TIWARI B. K., VALDRAMIDI V. P., O'DONNELL C. P., MUTHUKUMARAPPAN K., BOURKE P., and CULLEN P. J. Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry*, 2009, 57: 5987–6000. https://doi.org/10.1021/jf900668n
[35] CARMICHAEL J., DEGRAFF W. G., MINNA J. D., and MITCHELL J. B. Evaluation of a tetrazolium based semi-automated colorimetric assay: assessment of chemosensitivity testing. *Cancer Research*, 1987, 47(4): 936. https://pubmed.ncbi.nlm.nih.gov/3802100/
[36] ALLEY M. C., SCUDIERE D. A., MONKS A., HURSEY M. L., CZERWINSKI M. J., FINE D. L., ABBOTT B. J., MAYO J. G., SHOEMAKER R. H., and BOYD M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Research*, 1988, 48: 589-601. https://pubmed.ncbi.nlm.nih.gov/3335022/

参考文:

[1] DUBAR F., KHALIFE J., BROCARD, J., DIVE, D., 和 BIOT C. 铁喹，一种巧妙的抗疟药—作用机制的思考。分子，2008，13: 2900-2907. https://doi.org/10.3390/molecules13112900
[2] COX-SINGH J., 和 SINGH B. 诺尔斯疟疾：新出现的疾病，具有公共卫生重要性。寄生虫学的趋势，2008，24: 406-410. https://doi.org/10.1016/j.pt.2008.06.001
[3] WHO. 2010年世界疟疾报告：世界卫生组织全球疟疾计划。世界卫生组织，日内瓦, 2010.
[4] PHILLIPSON J. D., 和 O'NEILL M. J. 抗疟和杀阿米巴天然产物。在：HOSTETTMANN K., & LEA P. J.（编辑）生物活性天然产品。牛津，克拉伦登出版社，1987: 49-64.
[5] SAXENA S. P. N, JAIN D. C., 和 BHAKUNI R. S. 植物来源的抗疟剂。当代科学，2003，85(9): 1314-1329. https://www.jstor.org/stable/24108135
[6] KAUR K., JAIN M., KAUR T., 和 JAIN R. 来自大自然的抗疟药。生物有机和药物化学, 2009, 17(9): 3229–3256. https://doi.org/10.1016/j.bmc.2009.02.050
[7] BRUCE-CHWATT L. J. 青蒿素：一种新型抗疟药。英国医学杂志，1982，284: 767–768. https://doi.org/10.1136/bmj.284.6318.767
[8] NOEDL H., SE Y., SCHAECHER K., SMITH B. L., SOCHEAT D., 和 FUKUDA M. M. 柬埔寨西部存在青蒿素耐药性疟疾的证据。新英格兰医学杂志，2008，359(24): 2619–2620. https://doi.org/10.1056/nejmc0805011
[9] GANESH D., FUEHRER H.P., STARZENGRÜBER P., SWOBODA P., KHAN W.A., REISMANN J. A. B., MUELLER M. S. K, CHIBA P., 和 NOEDL. H. 孟加拉国黄酮醇槲皮素的抗疟原虫活性。寄生虫学研究，2012，110: 2289–2295. https://doi.org/10.1007/s00436-011-2763-z
[10] DHAR R., ZHANG K., TALWAR G. P., GARG S., 和 KUMAR N. 印楝（印楝）级分抑制人类疟原虫恶性疟原虫药物敏感和耐药菌株的无性和有性阶段的生长和发育。民族药理学杂志，1998，61(1): 31–39. https://doi.org/10.1016/s0378-8741(98)00012-9
[11] WERNSDORFER W. H., 和 TRIGG, P. I. 疟疾研究的最新进展：化疗。在：WERNSDORFER W. H., & MCGREGOR I.（编辑）疟疾原理和疟疾学实践。丘吉尔利文斯顿，爱丁堡, 1988: 1569–1674.
[12] PRESBER W., HEGENSCHEID B., HERNANDEZ-ALVAREZ H., HERMANN D., 和 BRENDEL C. 安哥拉安哥拉铜草提取物在体外抑制恶性疟原虫和伯氏疟原虫的生长。热带学报，1992，50: 331–338. https://doi.org/10.1016/0001-706x(92)90068-9
[13] GESSLER M. C., NKUNYA M. H. H., MWASUMBI L. B., HEINRICH M., 和 TANNER M. 筛选坦桑尼亚药用植物的抗疟活性。热带学报，1994，56: 65–77. https://doi.org/10.1016/0001-706x(94)90041-8
[14] FRANSSEN F. F. J., SMEIJSTERS L. J. J. W., BERGER I., 和 ALDANA, B. E. M. 危地马拉传统上用于对抗疟疾的一些植物的体内和体外抗疟原虫活性。抗菌药物和化疗，1997，41: 1500–1503. https://doi.org/10.1128/aac.41.7.1500
[15] MACKINNON S., DURST T., ARNASON J. T., ANGERHOFER C., PEZZUTO J., SANCHEZ-VINDAS P. E., POVEDA L. J., 和 GBEASSOR M. 热带楝科提取物和葛杜宁衍生物的抗疟活性。天然产物杂志, 1997, 60: 336–341. https://doi.org/10.1021/np9605394
[16] JENETT S. K., FRANK P. M., ULRICH B., MAHABIR P. G., 和 ECKART E. 中美洲药用植物的体外抗疟原虫活性。热带医学和国际健康，1999，4(9): 611–615. https://doi.org/10.1046/j.1365-3156.1999.00456.x
[17] KRETTLI A. U. 抗疟药物发现：巴西药用植物和纯化化合物的筛选。药物发现的专家意见，2009，4: 95-108. https://doi.org/10.1517/17530050802678127
[18] NOEDL H., ATTLMAYR B., WERNSDORFER W. H., KOLLARITSCH H., 和 MILLER R. S. 一种用于现场使用的富含组氨酸蛋白2的疟疾药物敏感性测定。美国热带医学与卫生杂志，2004，6(71): 711–714. https://pubmed.ncbi.nlm.nih.gov/15642959/
[19] MOSMANN T. 细胞生长和存活的快速比色测定：在增殖和细胞毒性测定中的应用。免疫学方法杂志，1983，65: 55-63. https://doi.org/10.1016/0022-1759(83)90303-4
[20] AYISI N. K., OPONG R. A., GYAN B, BUGYEI K., 和 EKUBAN F. 恶性疟原虫：通过基于四唑的比色测定评估氯喹、心叶藻、抛光的无花果和其他药物的作用选择性。疟疾研究与治疗, 2011: 816250. https://doi.org/10.4061/2011/816250

[21] ADEDAPO A, JIMOH F., 和 AFOLAYAN A. 鬼针草和藜叶的丙酮、甲醇和水提取物的营养价值和生物活性比较。波兰药学学报, 2011, 68(1): 83-92. https://pubmed.ncbi.nlm.nih.gov/21485705/
[22] SOOD P., MODGIL R., SOOD M., 和 CHUHAN P. K. 不同藜属品种叶子的抗营养特性。食品科学与技术年鉴, 2012, 13(1): 68-74. https://afst.valahia.ro/wp-content/uploads/2022/09/3-PurvikaSood_2012.pdf
[23] ARORA S. K., ITANKAR P. R., VERMA P. R., BHARNE A. P., 和 KOKARE D. M. NFκB 参与藜的地上部分提取物的抗风湿潜力。民族药理学杂志, 2014, 155(1): 222-229. https://doi.org/10.1016/j.jep.2014.05.026
[24] AGARWAL S. S., YAMREKAR B. P., 和 PARIDHAVI M. 临床有用的草药。阿胡贾出版社，新德里, 2005: 10-12.
[25] KHARE C. P. 印度药用植物。施普林格国际出版社，新德里, 2007: 141-142.
[26] PRIYA S., YOGESH S., SINGHAI A. K., 和 ABHISHEK S. 藜的药理和植物化学特征。药学与技术研究杂志, 2010, 3(4): 960-963.
[27] BALDI A., 和 CHOUDHARY N. K. 藜提取物的体外抗氧化和保肝潜力。国际绿色药学杂志, 2013, 7(1): 50-56. http://www.greenpharmacy.info/index.php/ijgp/article/view/297
[28] GOGOI B., 和 ZAMAN K. 阿萨姆邦“博哈格· 比胡”期间配方中使用的一些药用植物物种的植物化学成分。生药学和植物化学杂志, 2013, 2(2): 30-40. https://www.phytojournal.com/vol2Issue2/3.1.html
[29] PAL A., BANERJEE B., BANERJEE T., MASIH M., 和 PAL K. 藜的保肝活性。植物对抗扑热息痛引起的大鼠肝损伤。国际药学和药物科学杂志, 2013, 3: 55-57. https://innovareacademics.in/journal/ijpps/Vol3Suppl3/2065.pdf
[30] CUTILLO F., DELLA GRECA M., GIONTI M., PREVITERA H., 和 ZARRELLI A. 来自藜属的苯酚和木酚素。植物化学分析, 2006, 17(5): 344-349. https://doi.org/10.1002/pca.924
[31] JHADE D., PADMAA M. P., 和 USHA G. 从藜属叶子中分离植物成分。药学研究杂志, 2009, 2(7): 1192-1193. https://www.researchgate.net/publication/40765427_Isolation_of_Phytoconstituents_from_the_leaves_of_Chenopodium_album_Linn
[32] BURT S. 精油：其抗菌特性及其在食品中的潜在应用-综述。国际食品微生物学杂志, 2004, 94: 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
[33] FRIEDMAN M., HENIKA P. R., LEVIN C. E., 和 MANDRELL R. E. 植物精油及其成分对苹果汁中大肠杆菌0157:H7和肠沙门氏菌的抗菌活性, 2004, 52: 6042–6048. https://doi.org/10.1021/jf0495340
[34] TIWARI B. K., VALDRAMIDI V. P., O'DONNELL C. P., MUTHUKUMARAPPAN K., BOURKE P., 和 CULLEN P. J. 天然抗菌剂在食品保鲜中的应用。农业与食品化学杂志, 2009, 57: 5987–6000. https://doi.org/10.1021/jf900668n
[35] CARMICHAEL J., DEGRAFF W. G., MINNA J. D., 和 MITCHELL J. B. 基于四唑的半自动比色测定的评估：化学敏感性测试的评估。癌症研究, 1987, 47(4): 936. https://pubmed.ncbi.nlm.nih.gov/3802100/
[36] ALLEY M. C., SCUDIERE D. A., MONKS A., HURSEY M. L., CZERWINSKI M. J., FINE D. L., ABBOTT B. J., MAYO J. G., SHOEMAKER R. H., 和 BOYD M. R. 使用微培养四唑测定对人类肿瘤细胞系组进行药物筛选的可行性。癌症研究, 1988, 48: 589-601. https://pubmed.ncbi.nlm.nih.gov/3335022/