

# Protection of DNA during oxidative stress and cytotoxic potential of *Artemisia absinthium*

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**Abstract:** Medicinal plants are rich in secondary metabolites (alkaloids, glycosides, coumarins, flavonoids, steroids, etc.) and considered to be more effective and a safer alternative source to manage a variety of diseases related to liver, heart and kidney disorders. This study determines *in vitro* antioxidant and *in vivo* toxicological profile including hemolytic, brine shrimp lethality and mutagenicity of aerial parts of *Artemisia absinthium*. DNA protection assay was performed on pUC19 plasmid vector using H<sub>2</sub>O<sub>2</sub> as oxidative agent. Total phenolic and flavonoid content were determined using colorimetric methods. Toxicity of the plant was evaluated by brine shrimp lethality, hemolytic and mutagenic activity. DNA protection assay of the plant showed concentration dependent protective effect and at concentration 10 μL/mL revealed complete protective effect against H<sub>2</sub>O<sub>2</sub> induced DNA damage. Highest phenolic and flavonoid content was found to be 167.3 (mg GAE 100g DW<sup>-1</sup>) and 14 (mg CE 100g DW<sup>-1</sup>) respectively. Results showed that *A. absinthium* is potent against standard toxicological procedures, that indicates the presence of bioactive components in the plant and possess antioxidant activity that protects DNA against H<sub>2</sub>O<sub>2</sub> induced oxidative damage. Thus the results showed/support that *A. absinthium* provides significant health benefits.

**Keywords:** Oxidative damage, Hemolytic, Mutagenic, pUC19 DNA, DPPH.

## INTRODUCTION

The use of medicinal plants to cure certain diseases has been practiced in many parts of the world since centuries. However limited data about the cytotoxicity and DNA protection is so far available. *Artemisia absinthium* commonly known as “Wormwood” (family; Asteraceae), has been used as herbal medicine throughout Asia, Middle East, North Africa, and Europe (Sharopov *et al.*, 2012). *A. absinthium* has a variety of biological uses including antiseptic, restoration of declining mental function, antispasmodic, cardiac stimulant, inflammation of the liver and to improve memory against acute liver injury (Amat *et al.*, 2010, Ahmed *et al.*, 2012). Phytochemical analysis of *A. absinthium* revealed the occurrence of important chemical compounds such as carotenoids, flavonoids and phenolic compounds (Canadanovic-Brunet *et al.*, 2005). Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, cancer, diabetes and cirrhosis (Jamuna *et al.*, 2011). Almost all organisms possess antioxidant defense systems and DNA repair mechanisms that have evolved to protect themselves against oxidative damage although these systems are insufficient to prevent the damage entirely (Ross *et al.*, 2002). The need of the time is to establish the safety of *A. absinthium* to be used as medicine, therefore our work aimed to investigate the

protective effect and safety of the plant using various *in vitro* and *in vivo* standard toxicological procedures.

## MATERIALS AND METHODS

### *Plant extract*

*Artemisia absinthium* (aerial parts) were purchased from herbal store, authenticated by a plant taxonomist and the voucher specimen (85-14-2) was submitted to the “herbarium/collection at Department of Botany, University of Agriculture Faisalabad, Pakistan”. The plant material was cleaned, powdered and macerated in methanol for one week in an orbital shaker. The extract was filtered and concentrated under reduced pressure using rotary evaporator.

### *Phytochemical analysis*

Total phenolic (TPC) and total flavonoid content (TFC) were determined using standard procedures described by Ho *et al.* (2010).

### *DPPH free radical scavenging assay*

For the determination of % inhibition DPPH free radical scavenging assay method described by Ho *et al.* (2010) was used with some modifications.

### *Antioxidant activity by DNA protection assay*

Antioxidant activity of the plant extract was determined by DNA protection assay following the protocol described by Riaz *et al.* (2012).

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### Cytotoxicity studies

The cytotoxicity of the plant extract was tested through various standard procedures such as brine shrimps lethality (Haq *et al.*, 2012), hemolytic activity (Riaz *et al.*, 2012) and mutagenic activity (Razak *et al.*, 2011).

### STATISTICAL ANALYSIS

Statistical analysis was done using statistical software and expressed as means  $\pm$  S.E.M. Values of  $p < 0.05$  were considered as significant.

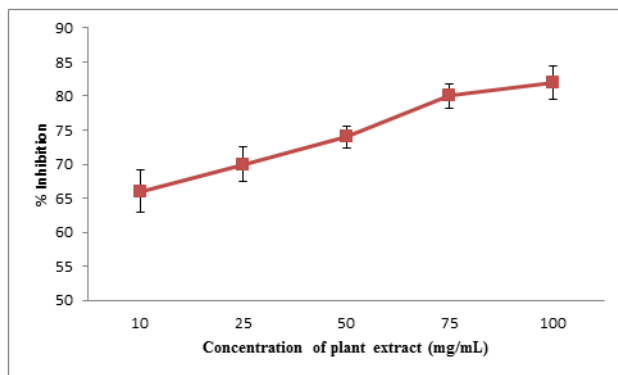
### RESULTS

#### Phytochemical analysis

The total phenolic and flavonoid content were found to be  $167.3 \pm 0.8$  (mg/g GAE) and  $14 \pm 0.1$  (mg/g CE) respectively. *A. absinthium* extract revealed high antioxidant activity against a variety of free radicals. We observed that a change in extract concentration affected the degree of antioxidant activity during experiment, hence it can be concluded that the antioxidant activity depends on the extract concentration.

#### DPPH scavenging activity

The aerial parts extract of wormwood was able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine. In this study, the extracts exhibited a concentration dependent antiradical activity by inhibiting DPPH radical (fig. 1).

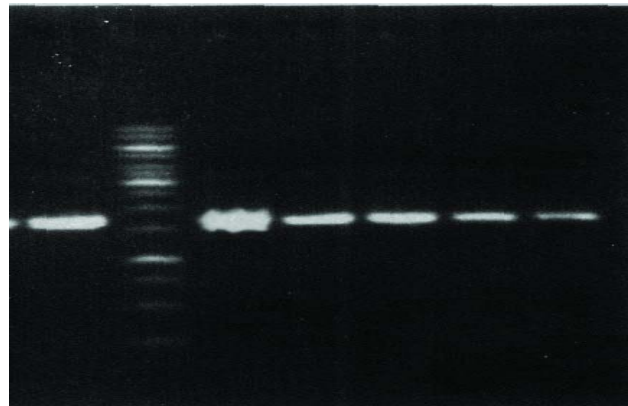


**Fig. 1:** Percentage inhibition of *A. absinthium* (aerial parts) at different concentration by DPPH

#### Protective effect of $H_2O_2$ induced oxidative damage on pUC19 DNA

DNA protection assay was performed to study the effect of free radicals generated by  $H_2O_2$  on super coiled pUC19 DNA in the presence and absence of plant extract in different concentrations shown in fig. 2. DNA without any kind of treatment remained intact in its super coiled form (lane 1). The hydroxyl radical of  $H_2O_2$  damaged the pUC19 DNA and resulted in streaking band due to strand cleavage (lane 3). *A. absinthium* at  $10 \mu\text{L/mL}$  concentration showed complete protective effect (lane 5) because in the presence of  $H_2O_2$  there were no sign of

degradation in DNA. As the concentration of plants extract increased its protective effect gradually decreased and moderate protective effect was observed at  $100 \mu\text{L/mL}$  and poor at  $1000 \mu\text{L/mL}$  concentrations (lane 6 & 7) because disruption in DNA was observed on both 100 and 1000.



**Fig. 2:** Agarose gel electrophoresis pattern of pUC19 plasmid DNA treated with 30mM  $H_2O_2$  in the presence and absence of different concentrations of plants extracts

Lane 1: Normal pUC19 DNA, Lane 2: DNA Ladder (1 kb, Fermentas), Lane 3: pUC19 DNA + 30mM  $H_2O_2$ , Lane 4: pUC19 DNA + *A. absinthium* [ $100 \mu\text{L/mL}$ ], Lane 5: pUC19 DNA + 30mM  $H_2O_2$  + *A. absinthium* [ $10 \mu\text{L/mL}$ ], Lane 6: pUC19 DNA + 30mM  $H_2O_2$  + *A. absinthium* [ $100 \mu\text{L/mL}$ ], Lane 7: pUC19 DNA + 30mM  $H_2O_2$  + *A. absinthium* [ $1000 \mu\text{L/mL}$ ].

#### Hemolytic activity

Hemolytic activity could be used as a primary tool for studying the toxicity of plant extract as it provides primary information on the interaction between molecules and biological entities at cellular level. The plant extract treatment of human erythrocytes caused very less hemolysis that represents the non-toxic effect of the extract towards human erythrocytes. The hemolytic activity of *Artemisia absinthium* at different concentrations was  $3 \pm 0.25$ ,  $4 \pm 0.50$ ,  $6 \pm 0.10$  and  $8 \pm 0.20$  at 10, 25, 50 and 100 mg.

#### Brine shrimp lethality

The toxicity of *A. absinthium* extract was evaluated using brine shrimps lethality test. The live larvae were counted after 24 hours and the percentage mortality was calculated (table 1). The percentage mortality observed through brine shrimps lethality was concentration dependent, lowest mortality was 20% and highest mortality 26% at 10 mg and 100 mg respectively.

#### Mutagenic activity

Mutagenic activity was performed by using test strains *S. typhimurium* TA 98 and *S. typhimurium* TA 98. In standard of both *S. typhimurium* TA 98 and TA 100

**Table 1:** Brine shrimp lethality outcomes showing survived shrimps and % mortality after treatment with extract of *A. absinthium*

Fraction	No. of survived shrimps in following intervals									% Mortality
	Initial	3 hour	6 hour	9 hour	12 hour	15 hour	18 hour	21 hour	24 hour	
Control	15	15±0	15±0	15±0	15±0	15±0	15±0	15±0	15±0	No activity
10 mg	15	14.5±0.5	14±0	13.5±0.5	13±0	12.5±0.5	12±0	12±0	12±0	20.0
25 mg	15	15±0	14±0	14±0	13.5±0.5	13±0	13±0	12.5±0.5	12±0	20.0
50 mg	15	14±0	13±0	13±0	12.5±0.5	12±0	12±0	11.5±0.5	11±0	26.0
100 mg	15	14±0	14±0	13.5±0.5	13±0	12.5±0.5	12±0	11±0	11±0	26.0

**Table 2:** Mutagenic activity of methanolic extract

Plant	No. of positive wells / total no. of wells		Interpretation
	<i>S. typhimurium</i> TA98	<i>S. typhimurium</i> TA100	
Background	21 / 96	13 / 96	
Standard	96 / 96	88 / 96	Mutagenic
<i>Artemisia absinthium</i>	1 / 96	1 / 96	Non-mutagenic

+, significant increase in the number of positive wells compared to the related control ( $p < 0.05$ ); -, no significant effect observed.

showed significant increase in number of positive wells. The numbers of positive wells in background of *S. typhimurium* TA 98 were 21/96 and 13/96 in *S. typhimurium* TA 100. The plant extract were non mutagenic and non-toxic to positive test strain (table 2).

## DISCUSSION

Oxidative stress is one of the causes for the development and progression of certain life threatening diseases such as cancer, hepatotoxicity, diabetes, atherosclerosis, hyperlipidemia, neuronal degeneration (Rajkumar *et al.*, 2010). Antioxidants from plant sources may be useful in prevention of such ailments as well as for treatment. Flavonoid and phenolic are natural antioxidant molecules that exhibit different therapeutic applications and pharmacological approaches. Significantly lower values of TPC 131.18 (mg 100g DW<sup>-1</sup>) and TFC 41.21 (mg 100g DW<sup>-1</sup>) were reported in *A. absinthium* (Lee *et al.* (2013). Whereas the alcoholic extract of another species of this genus, contained total phenolic content (152.8 mg 100/g DW<sup>-1</sup>) and flavonoid content (109.20mg 100/g DW<sup>-1</sup>) (Gouveia and Castilho, 2011; Carvalho *et al.*, 2011).

The positive correlation between polyphenolic content of the extract and its antioxidant activity is well documented (Huang and Mau, 2006). Therefore, the content of total phenolic compounds in the extract might explain their high antioxidant activities. Highest antioxidant activity of *A. absinthium* extract was 82.31% at 100 that designates strong radical-scavenging capacity. Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxides (Li *et al.*, 2007). The DPPH values in aqueous extract were

found slightly different in another study whereas similar radical scavenging activity was observed in methanol extract of *A. absinthium* (Lee *et al.*, 2013). Our study clearly indicates that the extract of *A. absinthium* is capable of inhibiting lipid peroxidation thus shield humans against infection and degenerative diseases.

DNA protective potential of the extracts can be exploited to develop the drugs to control cancer. Phenolic compounds are well established antioxidant compounds reported from plants and vegetables (Pandey and Rizvi 2009). Results showed a concentration dependent antioxidant ability of medicinal plants. Reactive oxygen species (ROS) could be the reason to damage the cellular genetic material. Free radicals could be the cause of DNA strand disruptions, only if the sugar moiety was damaged and usually two main types of such changes are observed in DNA at the molecular level which are altered bases and strand breaks. Both types of changes, if not repaired, affect the cell structure and function. The protective effect of medicinal plants on DNA could be explained by its ability to scavenging ROS due to its property as an antioxidant (Yoshikawa *et al.*, 2006).

In present experiment, DNA damage by free radicals generated by H<sub>2</sub>O<sub>2</sub> is observed whereas in another study, DNA was damaged by e-radiations and protective effect of hesperidin was investigated. The findings of DNA protection were similar either DNA is damaged due to free radicals or e-radiation. The protective effect was concentration dependent in hesperidin as well as in *A. absinthium*. Hosseinimehr and Nemati (2006) reported that hesperidin protected mice against concentration dependent radiation induced DNA damage in bone marrow cells whereas in this study similar results were observed.

In another study Rajkumar *et al.* (2011) reported that the aqueous extract of *Rheum emodi* displayed considerable protective activity in comparison to the methanolic extract which did not show any protective activity. In their study UV-photolysis as well as H<sub>2</sub>O<sub>2</sub> treatment damaged the entire DNA. They concluded that UV photolysis and H<sub>2</sub>O<sub>2</sub> treatment of pBR322 obliterated the entire DNA, while 50µg of the plant aqueous extract gave partial protection against DNA damage. Similarly protection of pUC19 DNA was observed against H<sub>2</sub>O<sub>2</sub> that indicates the extracts of *A. absinthium* are capable of inhibiting lipid peroxidation. The possible mechanism is by scavenging the free radicals and preventing hydroxyl radicals from attacking lipids. In addition, DNA protection assay also supports the hydroxyl radical scavenging activity of the studied plants extracts.

The hemolytic activity was higher in another plant species *Aerva lanata* than *A. absinthium* (Gaurav *et al.* (2013). In another study Yinebeb *et al.* (2011) also reported weak hemolytic effect (0.8-19.2) against the THP-1 cell line from *A. Absinthium* oil.

The hemolytic effect of *A. Absinthium* was less than other fractions, however, data obtained are in a safe range and the plant extracts may be safe for use as medicine.

*Artemia* bioassay has been demonstrated to provide a viable alternative to the mouse bioassay, which is expensive and associated with ethical constraints (Kohler *et al.*, 2002). The results described above clearly indicate that aerial parts of *A. absinthium* can be used as alternative source to manage various ailments.

Mulaudzi *et al.* (2013) studied mutagenic activity of medicinal plants used against various diseases by Venda people and found that all the plant extracts were non-mutagenic towards *Salmonella typhimurium* strain TA98 except for *Elephantorrhiza burkei* and *Ekebergia capensis* that showed weak mutagenicity. *Ekebergia capensis* bark induced 50.0 revertant colonies at 500mg/mL and 50mg/mL. The mutagenic activity was not dose dependent.

In another study Sarac and Sen (2014) performed anti-mutagenicity assays of Liquid *ambar orientalis* using TA 98 and TA100 strains and evaluated the extract for anti-mutagenic activity at 2.5, 0.25 and 0.025 mg/plate concentrations and found strongest anti-mutagenic activity at 2.5 mg/plate concentration against *S. typhimurium* TA 98 strain, only one concentration (0.025 mg/plate) of the extract did not exhibit any anti-mutagenic effect against *S. typhimurium* TA 100. The results shows that extract had a better anti-mutagenic effect (37.7–85.67%) on the TA98 strain. It was observed that anti-mutagenic activity is dose dependent. The freshly collected *Acokanthera oppositifolia* plant extract at 5000µg/mL exhibited mutagenic effects against TA1535 strain against three bacterial strains reported by Aremu *et al.* (2013). Whereas Mortelmans and Zeiger, found explored that mutagenic potential observed with TA1535

strain is associated with the substitution of leucine with proline in the bacterial genome. Mostly, a positive response in any single bacterial strain either with or without metabolic activation is adequate to designate a substance as a mutagen (Zeiger, 2001).

In another study mutagenic and anti-mutagenic activities of aqueous and methanol extracts of *Euphorbia hirta* and found that Quercetin (25g/mL) have strongly mutagenic in *S. typhimurium* TA 98 in the absence and presence of S-9 metabolic activation. Whereas both the methanol and aqueous extracts at concentration up to 100g/mL in the absence and presence of S-9 metabolic activation were non-mutagen when tested with *S. typhimurium* TA98 and TA 100 strains Loh *et al.* (2009).

## CONCLUSION

*Artemisia absinthium* extract showed significant antioxidant potential in terms of scavenging free radicals thus shield humans against infection and degenerative diseases. The H<sub>2</sub>O<sub>2</sub> induced oxidative damage in plasmid pUC19 DNA was evaluated and it was found that methanolic extract of *A. absinthium* protected the DNA, which may be due to the presence of phytochemicals that confirmed its antioxidant properties. The hemolytic activity against human RBCs showed a minor cytotoxicity that may be used in herbal medicine.

## ACKNOWLEDGEMENTS

The authors express their deep sense of gratitude to Dr. Muhammad Shahid and Dr. Mazhar Abbas for their guidance and support.

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