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Evaluation of *In-Vitro* Antioxidant, Anti-inflammatory Properties of Aerial Parts of *Zanthoxylum rhesta.*

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ABSTRACT

Zanthoxylum rhesta is used as a traditional medicine in many parts of the world. The aim of the present study was to determine the invitro antioxidant activity by DPPH and ABTS free radical scavenging methods. *Z.rhesta* aerial parts were extracted with hexane, ethyl acetate, mehthanol and water. Each extract was subjected to DPPH and ABTS radical scavenging activity, total phenolic content, flavonoids and reducing power determination. Ethyl acetate, methanol and water extracts showed significant DPPH and ABTS free radical scavenging activity. Methanol extract had highest amount of phenolic and flavonoids contents followed by ethyl acetate and methanol extracts. The reducing power was also found to be maximum in methanol extract. Hexane extract did not show any biological activity. The medicinal activity of the ethyl acetate, methanol and water extract validated the traditional claims of this plant for the treatment of various ailments.
Keywords: antioxidant, anti-inflammatory, medicinal plant, *Zanthoxylum rhesta*

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INTRODUCTION

Normal metabolism and some external factors induce the production of free radicals such as, superoxide anions, hydrogen peroxides, hydroxyl radicals etc. Excess generation of reactive species leads to oxidative stress. The resulting oxidative stress causes various diseases such as, cancer, atherosclerosis, diabetes mellitus, renal failure, ageing and etc. Antioxidants are the substances that prevent oxidative stress at lower concentrations. Most of the commercially available antioxidants are synthetic ones and they have the drawback of side effects when taken in vivo. Secondary metabolites produced by the plants are the rich source of natural antioxidants and they include carotinoids, flavonoids phenolics and ascorbic acids. These chemicals are produced by the plants for their sustenance. These antioxidants have the capability to delay or prevent the diseases caused by oxidative stress without any side effects. So, the search for plant derived antioxidant is gaining the attention of many researchers and medical practitioners [1-5].

The *Zanthoxylum rhetsa* (Roxb.) DC. belongs to the family Rutaceae. Different parts of this plant are used in traditional medicine as a therapy for diabetes, spasmodic, diuretic and inflammatory complications [6]. Kanikkar and Naga tribes in India use this plant to relieve pain, increase lactation in nursing mothers and as a deworming remedy respectively [7-8]. *Z. rhetsa* contains a terpenoid, xanthyletin and sesamin, alkaloids, flavonoids and sabinene [9-11,6]. Significant antinociceptive, antidiarrheal and anti infectant activities has been reported in this plant [12,7]. However, there are no reports available on the antioxidant potential Hence, the present study was undertaken to estimate the antioxidant activity, total phenolic , flavonoid content and reducing power of different extracts of aerial parts of *Z. Rhetsa.*

MATERIALS AND METHODS

1,1-Diphenyl-2-picryl hydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiozoline-6-sulphonic acid) diammonium salt(ABTS), were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dimethylsulphoxide (DMSO) Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Sodium carbonate (Na2CO3), Rutin and Folin-Ciocalteu were from Merck (India) Ltd, India. All other chemicals used in the study were obtained commercially and were of analytical grade.

Plant material

Aerial parts of *Z. rhesta* were collected in and around Udupi, Karnataka in the month of February 2014, washed with water thoroughly to remove the dust, and then washed with distilled water 3 to 4 times.The plant was authenticated by comparing the herbarium with the previously deposited herbariums in Department and the specimen was deposited in the Department of Botany, FMKMC College, Madikeri. Karnataka, India.

Preparation of plant extract

40g of the dried powder of *Z. rhesta* was successively extracted with (300 ml) hexane, ethyl acetate, methanol and water using Soxhelt apparatus. After the extraction, the extracts were filtered with the help of filter paper; the solvent was evaporated to get the dried extracts. The extracts were stored in refrigerator at 4^{o}C until use.

Phytochemical screening

Preliminary phytochemical screening of the crude extracts of the aerial parts was carried out with the standard methods with little modifications [13].

Determination of total phenolic content

The total phenolic compounds in the extract were determined according to the method of Singleton and Rossi as cited in Çoruh et al [14] with some modifications. To 1.0 ml of methanolic extracts, 5 ml of 2 % (w/v) sodium carbonate solution was added and vortexed vigorously. After 5 min, 0.5 ml of 1:1 diluted Folin Ciocalteu's phenol reagent was added and vortexed again. Same procedure was followed for the standard

solution of tannic acid (0.05-0.3 mg/ml). All the tubes were incubated at room temperature for 30 min and the absorbance was measured at 760 nm. The total phenolic content in the extracts were expressed as tannic acid equivalent in mg/g (TA mg/g).

Determination of total flavonoids

Total flavanoid content was measured according to the method of Re *et al* [15] with slight modifications. Rutin (100 µg/ml) was used as standard. Different extracts (1 mg/ml) and rutin were taken in different test tubes. Volume in each tube was made upto 4.0 ml with distilled water and 0.3 ml of 5% sodium nitrate was added. After 5 minutes, 0.3 ml of 10% aluminium chloride was added and incubated for 6 minutes at room temperature. 2 ml of 1M sodium hydroxide and 3.4 ml of distilled water were added to all the tubes, contents were mixed and the absorbance was measured at 517nm.

Determination of free radical scavenging activity by DPPH method

The free radical scavenging activity by DPPH method is based on the procedure of Brand- Williams *et al* [16] with modifications. Standard ascorbic acid (100µg/ml) and extracts (200µg/ml) were taken in different aliquots (0-1.0ml). Volume was made upto 1.0ml with methanol. 3.0 ml of 0.1M DPPH was added. Reaction mixtures were incubated at dark for 30 minutes and absorbance was measured at 517 nm. DPPH activity was calculated by using the following equation,

$$\text{Inhibition (\%)} = \left[\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \right] \times 100$$

Determination of free radical scavenging activity by ABTS method

The procedure is based on the method of Re *et al* [15] with few modifications. Stock solutions included 7.5mM $ABTS^{\cdot+}$ solution and 3mM potassium persulfate. The working solution was prepared by mixing equal volume of two stock solutions and allowing them to react for 12-16 hrs at room temperature in dark. The resultant intensively colored ABTS radical cation was diluted with 0.01M phosphste buffer saline (PBS), pH 7.4, to give an absorbance value of 0.70 ± 0.02 at 734 nm. Different aliquots standard (100µg/ml) and extracts (200µg/ml) were taken and made up to 1.0 ml with methanol. 2.0 ml of ABTS was added and the absorbance was measured at 734 nm.

$$\text{ABTS scavenging activity (\%)} = \left[\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \right] \times 100$$

Total Reducing power

Various fractions (0.1mL) were mixed with 2.5mL of 0.2M phosphate buffer (pH 6.6) and 2.5mL of 1 % potassium ferricyanide. After the mixture was incubated at 50ºC for 20 minutes, 2.5mL of 10 % trichloroacetic acid, 2.5mL distilled water and 0.5mL of 0.1% ferric chloride solution were added and the absorbance was measured at 700nm against a blank. The blank consists of all the reagents except the sample. Increasing absorbance of the reaction mixture indicates the increasing reducing power [17].

Statistical analysis

All experiments were performed in triplicate (n=3) and results were expressed as mean ± SEM. Statistical analysis was carried out with (Prism package version 3.0) using ANOVA ($P<0.05$).

RESULTS AND DISCUSSION

The hexane, ethyl acetate methanol and water extracts of *Z.rhesta* aerial parts were subjected to phytochemical analysis using standard protocol. Methanolic, ethyl acetate and water extract showed positive

results for phenolic compounds, saponins and flavanoids. Ethyl acetate and methanolic extracts showed positive results for alkaloids and tannins. Steroids were found to be absent in all the four extracts (Table 1).

Free radical scavenging activity was determined using DPPH and ABTS method. Ascorbic acid was taken as standard. Ethyl acetate, methanol and water extracts showed significant free radical scavenging activity in DPPH radical scavenging method (Figure 1). The IC_{50} values of ethyl acetate, methanol and water extract were found to be 47.34µg, 61.05µg and 66.40µg respectively (Table 2.)

Free radical scavenging activity of plant extracts can be determined by various methods. DPPH assay is one of the most widely used methods [17]. DPPH is a stable, nitrogen-centered free radical, which produces violet colour in ethanol solution. It was reduced to diphenylpicryl hydrazine, a yellow colored product. The color intensity of the product increases with the increase in the concentration of radical scavenging molecules. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. The significant DPPH scavenging potential of extracts of *Z.rhesta* aerial parts extracts may be due to hydroxyl groups present in the phytochemicals.

Significant DPPH radical scavenging activity of *Z.rhesta* extract instigated our curiosity to look for the ability these extracts to inhibit the formation of ABTS radical. ABTS radicals scavenging activity is predominantly used screen antioxidant activity of fruits, vegetables, foods and plants, and it has an advantage over other methods, as it is applicable to both lipophilic and hydrophilic antioxidants. This method has an added advantage, as the long wavelength absorption maximum at 734 nm eliminates color interference in plant extracts [18].

The ABTS radical scavenging potential was similar in all the extracts tested except ascorbic acid (Figure 2). IC_{50} value of ethyl acetate, methanol and water extract was found to be 56 µg, 60.93µg and 67µg respectively (Table 3).

The IC_{50} values of all the extracts of *Z.rhesta* aerial parts showed a difference in DPPH and ABTS radical scavenging activity measurement (Table 2 and 3). The observed difference is due to the action of these radicals at different levels [19].Similar results have been reported in other plants also [20].

The phenolic content in *Z.rhesta* extracts was determined by Folin-Ciocalteau method. The total phenolic content was expressed in terms of tannic acid equivalent. Methanol extract showed highest phenolic content and hexane extract did not show the presence of phenolics. The total phenolic content of ethylacetate, methanol and water extracts were found to be 258µg, 533µg and 349µg equivalents of tannic acid respectively (Figure 3).

Total phenolic content assay is employed routinely in studying phenolic antioxidants by using Folin-Ciocalteu reagent. It is a simple, convenient and reproducible method [21].The ubiquitous nature of plant phenolics and its therapeutic uses such as antioxidant, anti mutagenic and cardiovascular complication reducing capabilities, make them significant molecules in designing the drugs for various ailments [22].Our study is in agreement with reports published earlier[23].

The total flavanoid content was determined by $AlCl_3$ method. Rutin was used as standard. The total flavanoid content was expressed as rutin equivalent. The ethylacetate, methanol and water extracts contain, 290µ, 584µg and 166µg equivalent of rutin respectively. Hexane extract did not show the presence of flavonoids (Figure 4).

The hydroxyl group present in the flavonoids are responsible for the free radical scavenging potential of most of the plants. The flavonoids may act through either chelating or scavenging process. Significant antioxidant activity of ethyl acetate, methanol and water extracts *Z.rhesta* may be due the presence of poly phenols as well as flavonoids [24].

The total reducing power of the plant extract was determined by taking ascorbic acid as standard. Increased absorbance indicates higher reducing power. The reducing power activity of ethyl acetate, methanol

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and water extracts were, 78%, 89% and 68 %respectively. Hexane extracts did not show any reducing power (Figure 5).

In the reducing power assay, Fe^{3+} is reduced to Fe^{2+} by the donation of electrons present in the plant sample. The amount of Fe^{2+} can then be monitored by measuring the formation of Perl's blue at 700nm. Increasing absorbance indicates an increase in reductive ability. This result correlates with the reducing power of the plant extracts, reported earlier [25].

Table 1: Phytochemical analysis of the different extracts of *Z.rhesta* aerial parts.

Phytochemical/s	HE	EE	ME	WE
Saponins	-	+	+	+
Phenolic compounds	-	+	+	+
Flavanoids	-	+	+	+
Glycosides	-	-	+	-
Carbohydrates	-	-	+	+
Tannins	-	+	+	-
Alkaloids	-	+	+	-
Steroids	-	-	-	-

Results are expressed as mean± SEM of three parallel measurements. HE=Hexane extract, EE= Ethyl acetate extract, ME= Methanol extract, WE= Water extract.

Table 2: IC_{50} values of different extracts of *Z.rhesta* aerial parts and ascorbic acid in DPPH radical scavenging method.

SAMPLE	IC_{50} VALUE(µg)
Standard (Ascorbic acid)	22.32
Ethyl acetate extract	47.34
Methanol extract	61.05
Water extract	66.40

Table 3: IC_{50} values of different extracts of *Z.rhesta* aerial parts and ascorbic acid in ABTS radical scavenging method.

Sample	IC_{50} VALUE (µg)
Standard (Ascorbic acid)	11.76
Ethyl acetate extract	56.00
Methanol extract	60.93
Water extract	67.00

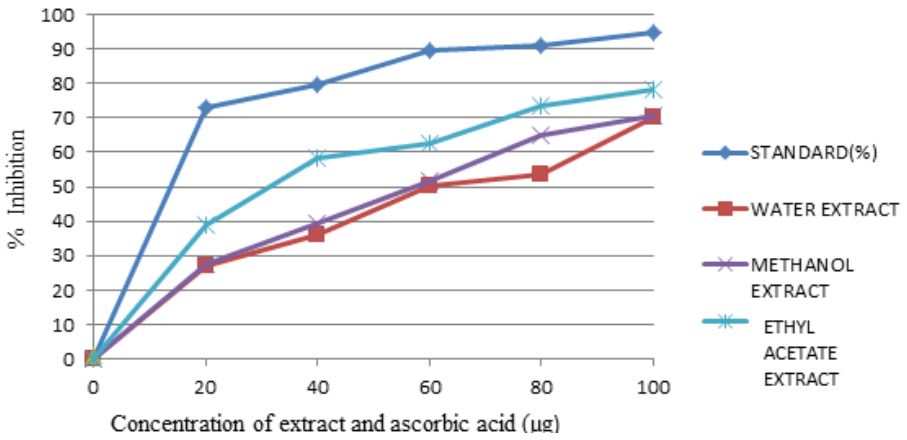

Figure 1: DPPH radical scavenging activity of *Z.rhesta* extracts and ascorbic acid.

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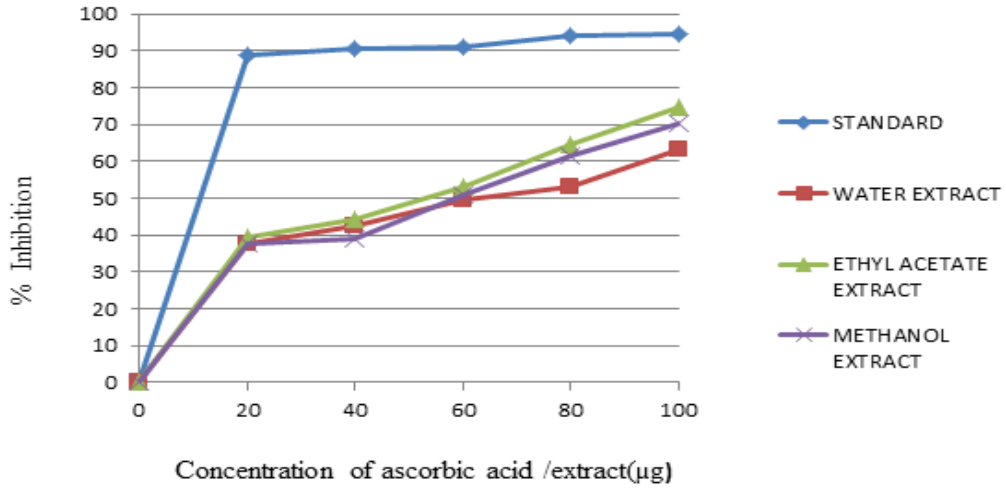


Figure 2: ABTS radical scavenging activity of *Z.rhesta* extracts and ascorbic acid.

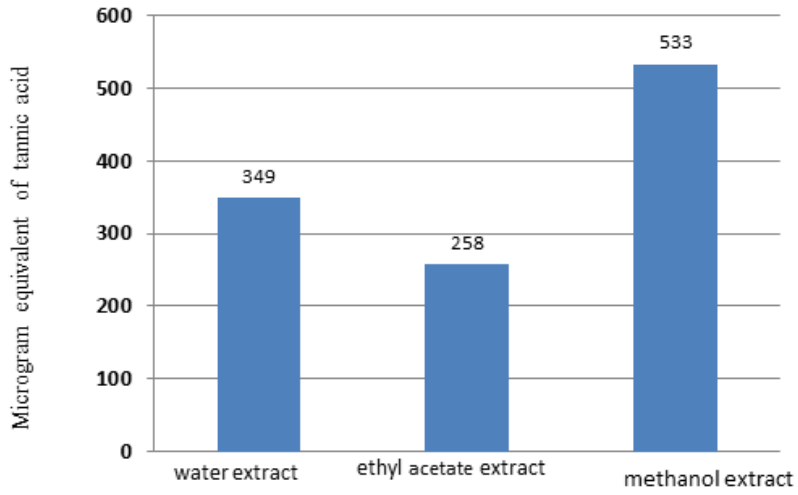


Figure 3: Phenolic content of *Z.rhesta* extracts.

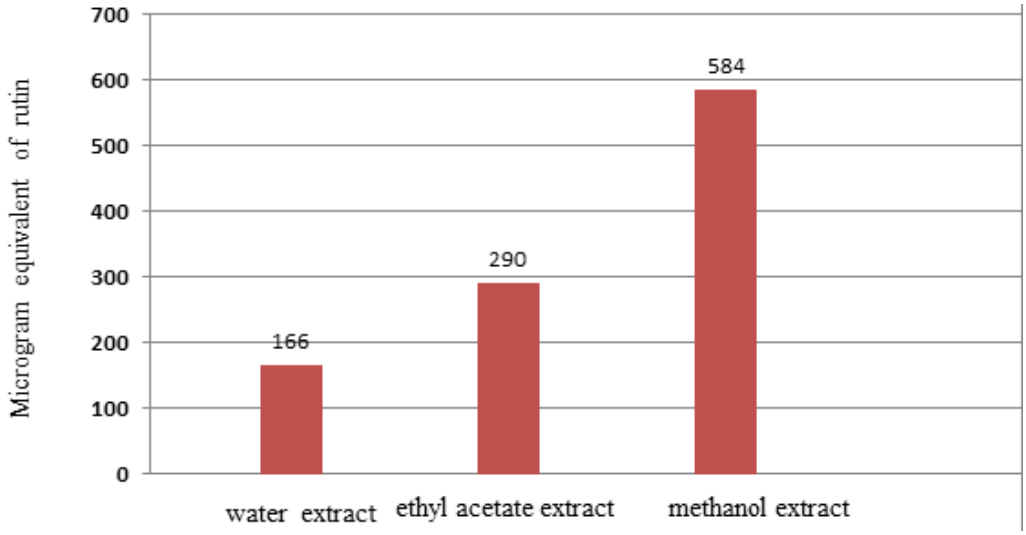
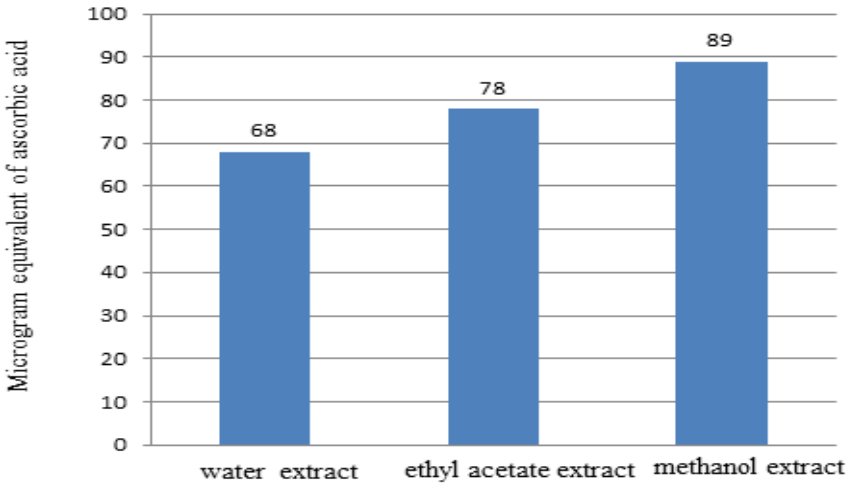


Figure 4: Flavonoid content in *Z.rhesta* extracts.

Figure 5: Total reducing power of *Z.rhesta* extracts.



CONCLUSION

We have reported for the first time about the antioxidant activity, total phenolic as well as total flavonoid content in the ethyl acetate, methanol and water extracts of *Z.rhesta* aerial parts. Medicinal plants with high flavonoid content are in great demand as they can cure many diseases associated with the oxidative stress. Further studies on isolation, characterization and the mechanism of action of lead molecule may help in the development of potent drug against various diseases associated with the oxidative stress with fewer side effects.

REFERENCES

[1] Pham-Huy LA, He H, Pham- Huy C. Int J Biomedical Sci 2008; 4(2): 89-96.
[2] Tiwari AK. Curr Sci 2004; 86(8):1092-1102.
[3] Halliwell B, Gutteridge JM C. Free radicals in biology and medicine (2nd ed.). Oxford: Clarendon press, 1989.
[4] Chen C, Pearson AM, Gray JI. Food Chem 1992; 43: 177-183.
[5] Mccall MR, Frei B. Free rad Biol and Med 1999; 26: 1034-1053.
[6] Pai V, Savadi RV, Bhandarkar A. Pharmacog J 2009; 1:33–36.
[7] Yadav AK, Tangpu V. J Parasit Dis 2009; 33(1&2):42–47.
[8] Lalitharani S, Mohan VR. J Herbal Med Toxicol 2010; 4: 191-192.
[9] Mathur RK, Ramaswamy SK, Rao AS, Bhattarcharya SC. Tetradron 1967; 23:2495–2498.
[10] Ahsan M, Zaman TA, Hasan CM, Ito C, Aslam SKN. Fitoterapia 2000; 71:697–700.
[11] Joy MT, Verghese J, Retamar JA, Talenti ECJ, Catalan CAN, Gros EG. Flav Fragran J 2006; 1:165–168.
[12] Rahman MT, Alimuzzaman M, Ahmad S, Chowdhury AA. Fitoterapia 2002; 73:340–342.
[13] Trease GE, Evans WC. Textbook of Pharmacognosy, 12th Edition, Balliere: Tindall,
[14] London, UK, 1989; 57-59, 343–383.
[15] Çoruh N, Sağdiçoğlu Celep AG, Özgökçe F, Đscan M. Food Chem 2007; 100(3): 1249-1253.
[16] Re R, Pellegrini M, Proteggente A, Pannala A , Yang M, Rice E C. Free Rad Biol
[17] Med 1999; 26(9-10): 1231–1237.
[18] Brand-Williams W, Cuvelier ME, Berset C. Food Sci and Tech 1995; 28(1):25-30.
[19] Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Free Rad Biol Med 1996; 21:895-902.
[20] Song F-L, Gan R-Y , Zhang Y, Xiao Q, Kuang L, Li HB. Int J Mol Sci 2010; 11(6):2362-2372.
[21] Dixit P, Ghaskabdi S, Mohan H, Devasayagam TP. Phytoher Res 2005; 19(11): 977-983.
[22] Joshi CG, Gopal M, Kumari S. Int J Cancer Res 2011; 7(4): 267-277.
[23] Huang D, Ou B , Proir RL. J Agric Food Chem 2005; 53(6):1841-1856.
[24] Yen GC, Duh PD, Tsai CL. J Agric Food Chem 1993; 41(1): 67-70.
[25] Joshi CG, Gopal M, Vaigundan D. J Chem Pharm Res 2011; 3(5):340-347.
[26] Das NP, Pereira TA. JAM Oil Chem Soc 67 1990; 67:255-258.
[27] Olayinka A, Aiyegoro, Okoh AI. Int J Mol Sci 2009; 10: 4990-5001.