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***RESEARCH ARTICLE***

# Optimization of Process Parameters for Total Phenol Extraction from Wood Waste using Response Surface Methodology

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## ABSTRACT:

The aim of present study is to optimize the important parameters of total phenol extraction form *Azadirachta indica* wood using ethanol. Experiments were conducted based on the central composite rotatable design (CCRD) and the models were constructed using response surface methodology (RSM). Response Surface Methodology (RSM) was employed for optimization of influencing factors such quantity of wood, temperature and extraction time in total phenol extraction using ethanol as solvent. The levels of mentioned parameters were in the range 1-5% of quantity of wood, 20-60°C of temperature and 1-5 days of extraction time were evaluated. The optimization of individual parameters were determined the 5% for quantity of wood, 50°C for temperature and 4 days for incubation time as central values. Based on the central values, 17 experiments were designed and experimentally conducted. The responses of 17 experiments were used for optimization. The best optimal condition of total phenol extraction was determined as quantity of wood 4.55 %, temperature 59.24°C and extraction time 3.92 days with extraction yield 0.958 mg Gallic acid equivalents (GAE)/g of dry powder. The phenolic extract quality was evaluated using thin layer chromatography and GC-MS analysis. The extract shows good anti-bacterial activity against *E. coli* and *S. aureus.*

**KEYWORDS:** Total phenol, CCRD, RSM, Thin Layer chromatography, Anti-bacterial.

## INTRODUCTION:

The earth was estimated to contain approximately a trillion tons of wood. In worldwide annual increment of wood is over 6000 million cubic meters. Man has utilized wood for several purposes since thousands of years. In addition to all the conventional usage in modern period wood was mainly used in the pulp and paper industry during last century[1]. Among the wood materials, lignocelluloses fraction of woodbased material was mostly used.

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However, the total wood biomass was utilization is only 2%[2]. Chemical composition of wood was mentioned in the Table 1

Wood biomass has seen to be a more effective carbon source of raw materials to replace the fossil fuels[3]. In future, many bio-refineries where effectively employed to transform biomass into chemicals and energy[4]. Some of the molecules derived from wood which includes resins and tannins were used since last 5 decades. Organic extractive includes fat materials, waxes, alkaloids, proteins, simple and complex phenolics, simple sugars, pectins, gums, resins, starches, saponins and essential oils. Wood biomass is considered to a rich source for various unique active molecules even today

science was not clearly discover biological character of those active molecules and its properties[5].

Phenolic compounds are plant secondary metabolites originated from the pentose phosphate, shikimate and phenyl propanoid pathways in plants[6]. The large group of phenolic compounds can be divided into two main subgroups: single phenols and polyphenols. In contrast to single phenols, polyphenols are characterized by the presence of more hydroxyl groups per molecule. Simple phenols have an aromatic ring with one or more hydroxyl groups; however, they can also carry some other substituents, e.g. methoxy- groups. Phenolic acids are simple phenols with one carboxyl group. Phenylpropanoic derivates have an aromatic system with 3 carbon side chains. This group includes cinnamic acids and their derivates, coumarins, and lignin. Flavone derivates are characterized by their flavone skeleton, which consists of two aromatic rings (A and B) and a heterocyclic ring with an oxygen atom in the middle[7].

The biosynthesis of the three above mentioned aromatic amino acids is best considered in two stages: the shikimate pathway from phosphoenolpyruvate and erythrose-4-phosphate to chorismate, which is common to phenylalanine, tyrosine, and tryptophan biosynthesis, and the three specific terminal pathways that use chorismate as a substrate. Because all three aromatic amino acids found in proteins are synthesized via the shikimate pathway, it has often been referred to as the common aromatic biosynthetic pathway.

Among the several extraction techniques solvent solid-liquid extraction, critical fluid, microwave assisted or combined methods of extraction yield more and more amount of phenolic contents. Overall scientific community want to extract the phenolic compounds out of plants in economically viable way which includes minimize the presence of toxic solvents, time consumption and so on. Even based on the above needs solid- liquid extraction was considered to be best where usage of steam distillation, low-pressure solvent along with microwave assistance or ultrasound assistance were used to improve the extraction process. Even high pressure solvent extraction all help to achieve greater yields[8]. Many plants have been evaluated for their hepatoprotective and antioxidant action in the light of modern medicine[9].

## MATERIALS AND METHODS:

### Collection of Neem Wood:

Neem wood dust was collected from nearby saw mills in Erode. Neem wood saw dust were dried for two days and stored in air tight containers for further uses.

### Total Phenol Extraction:

Total phenol content of Neem wood was extracted using Ethanol (60%) as solvent and the experimental parameter like Quantity of wood, Extraction time and Temperature were evaluated under One Variable at Time (OVAT) analysis to extracted total phenol present in the Neem wood.

### Quantification of Total Phenolic Content:

The total phenolic content (TPC) was determined according to the Folin-ciocalteu method using Gallic acid as a standard (the concentration range: 0.01 to 0.05 mg/mL). The test samples were taken in the volume of 0.9 and 1ml. To each sample 1.5 ml of Folin-ciocalteu reagent (50% v/v) was added and mixed. After 5 minutes, 4 ml of $Na_2CO_3$ (20 %, m/v) was added to the mixture and adjusted the volume test tube to 10mL using distilled water. After standing for 30 mins at room temperature, the absorbance was measured at 738 nm. Gallic acid samples were used to construct the standard curve. Comparing with standard curve, values of test samples can be determined. Total Phenolic Content was expressed as mg gallic acid equivalents (GAE)/g of dry powder.

### One Variable At Time (OVAT):

The phenolic extraction experiments were performed as batch process in a 100 ml of Erlenmeyer flasks Containing 20 ml of 60% ethanol as solvent. The wood dust was added to the flasks and incubated for extraction process. In the present study, three parameters which greatly influencing extraction process namely quantity of wood, extraction time and temperature were selected as variables for design of experiments. The parameters and the experimental conditions were tabulated in Table 1 for total phenol extraction.

**Table 1. Operational condition for total phenol extraction**

| Variables | Tested conditions | Fixed parameters | | |
|---|---|---|---|---|
| **Quantity of wood** | 1,2,3,4 and 5% w/v | Temperature30°C | Incubation time 1 day | 20 ml of 60% ethanol |
| **Temperature** | 20, 30, 40, 50 and 60,70°C | Incubation time 1 day | 5 g of wood | 20 ml of 60% ethanol |
| **Extraction time** | 1,2,3,4 and 5 days | 5 g of wood | Temperature 50°C | 20 ml of 60% ethanol |

## RESPONSE SURFACE METHODOLOGY:

Response Surface Methodology is an effective investigational tool used for developing and optimizing the process parameters with use of combination of strong mathematical and statistical techniques. If selected variables are assumed to be measurable, true functional relationship a second- order polynomial model is developed is RSM as follows[10].

$$Y = b0 + \sum_{i=1}^{n} biXi + \sum_{i=1}^{n} biiXiXi + \sum_{i=1}^{n} \sum_{i=1}^{n} bijXiXj + \varepsilon$$

Where X1,X2……Xn are the input factors that can control the response Y; n is the number of variables, b0 is the constant coefficient, bii is the quadratic coefficient, bij is the interaction of the coefficient and $\varepsilon$ denotes random error. By considering the variables are independent, continuous and experimentally controllable with negligible errors, the determination of a suitable approximation of a suitable approximation for response variable (Y) provides the optimal values of process parameters.

**Central composite rotatable design:**

The full factorial, partial factorial and central composite rotatable designs (CCRD) are the commonly used techniques, to explore the design of experiments (DOE). Hence, CCRD was chosen to design the experiments. The CCRD for this study consists of a16 ($2^n$, where n=4) full factorial design, (2*n) points fixed axially at a distance α and 6 center point at (0, 0,….,0). Thus the replicates of center level experiments are very important. The choice of α establishes the rotatability of a central composite design and α=2 for four factors. Hence, a total of Seventeen batch experiments were performed to satisfy a CCRD. The ranges of values of the selected variables are coded to lie at ±1 for the factorial levels, ±α for the axial levels and 0 for the center levels. The codes are calculated as a function of each factor as shown in Table 2.

**Table 2. Relationship between coded and actual values of variable**

| Code | Actual Value |
|---|---|
| +α | $X_{max}$ |
| +1 | $[(X_{max}+X_{min})/2]+[X_{max}-X_{min})]/(2\beta)]$ |
| 0 | $X_{max}+X_{min})/2$ |
| -1 | $[(X_{max}+X_{min})/2]-[X_{max}-X_{min})]/(2\beta)]$ |
| -α | $X_{min}$ |

**Determination of Central levels:**

Prior to follow DOE in the total phenol extraction process, the central levels of preferred parameters quantity of wood, temperature and extraction time were determined through One Variable at Time (OVAT) approach. Based on this approach, the level of any one independent parameter in changed at a time, while keeping other parameters as fixed. For that reason, the independent series of extraction process were carried out using 60% of ethanol as solvent.

**Experimental design:**

The total phenol extraction experiments were carried out in 100 ml Erlenmeyer flask containing 20ml of working solutions. CCRD was used to design the experiment. The ranges of values for each chosen variable in total phenol extraction process are given in Table 3.

**Table 3. Independent variables and their levels for CCRD for total phenol extraction**

| Variable | Symbol | Coded levels | | |
|---|---|---|---|---|
| | | -1 (low) | 0 (Center) | +1 (High) |
| Quantity of Wood (%) | A | 4 | 5 | 6 |
| Temperature (◦C) | B | 50 | 60 | 70 |
| Extraction time (days) | C | 3 | 4 | 5 |

**Table 4. Actual and coded levels of variables based on CCRD arrangement for total phenol extraction**

| Run | Actual Values | | | Coded values | | |
|---|---|---|---|---|---|---|
| | A (%) | B (°C) | C (days) | | | |
| 1 | 5.00 | 50.00 | 5.00 | 0 | -1 | +1 |
| 2 | 4.00 | 60.00 | 3.00 | -1 | 0 | -1 |
| 3 | 5.00 | 50.00 | 3.00 | 0 | -1 | -1 |
| 4 | 6.00 | 70.00 | 4.00 | +1 | +1 | 0 |
| 5 | 6.00 | 60.00 | 5.00 | +1 | 0 | +1 |
| 6 | 6.00 | 60.00 | 3.00 | +1 | 0 | -1 |
| 7 | 6.00 | 50.00 | 4.00 | +1 | -1 | 0 |
| 8 | 5.00 | 60.00 | 4.00 | 0 | 0 | 0 |
| 9 | 4.00 | 70.00 | 4.00 | -1 | +1 | 0 |
| 10 | 5.00 | 60.00 | 4.00 | 0 | 0 | 0 |
| 11 | 5.00 | 70.00 | 5.00 | 0 | +1 | +1 |
| 12 | 5.00 | 60.00 | 4.00 | 0 | 0 | 0 |
| 13 | 4.00 | 50.00 | 4.00 | -1 | -1 | 0 |
| 14 | 5.00 | 60.00 | 4.00 | 0 | 0 | 0 |
| 15 | 4.00 | 60.00 | 5.00 | -1 | 0 | +1 |
| 16 | 5.00 | 70.00 | 3.00 | 0 | +1 | -1 |
| 17 | 5.00 | 60.00 | 4.00 | 0 | 0 | 0 |

**Chromatographic Analysis of the Phenolic Extract**

**Thin Layer Chromatography:**

Ethanolic extract was subjected to Thin Layer Chromatography (TLC) as per conventional one dimensional ascending method using silica gel plate 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary tubes. In chamber contains solvent system as methanol and chloroform in the ratio of 4:1. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor ($R_f$), values were calculated for samples.

$$Rf = \frac{\textit{Distance travelled by the solute}}{\textit{distance travelled by the solven front TLC plates}}$$

**Gas Chromatography- Mass Spectrometry (GC-MS):**

The Ethanolic extract was subjected to Gas Chromatography- Mass Spectrometry Analysis at Sophisticated Instrumentation Facility, VIT University. GC-MS analysis was done using following procedure where Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5%

biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min−1; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. It measures the fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

### Anti-Bacterial Activity of Phenolic Extract:

Anti-bacterial activity extracted of Total phenol from *A.indica* wood using ethanol was evaluated against *Escherichia coli*,*Bacillus subtilis*, *Staphylococcus aureus* and *klebsiella pneumoniae*. *In vitro* agar diffusion assay was carried out using extracted total phenol against above organism in two different concentrations (50 and 100µl) and incubate overnight to examine it antimicrobial activity. The activity was measured using scale and expressed in mm.

## RESULTS AND DISCUSSION:

### Linearity Curve for Total Phenol Estimation:

**Table 5. Total phenol Estimation Assay**

| Constituents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ |
|---|---|---|---|---|---|---|
| **Volume of Gallic Acid (ml)** | 0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 |
| **Concentration of Gallic acid (µg/ml)** | 0 | 10 | 20 | 30 | 40 | 50 |
| **Volume of Folin's - ciocalteu reagent (ml)** | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| | Incubate at room temperature for 5 minutes | | | | | |
| **Volume of 20% $Na_2CO_3$ (ml)** | 4 | 4 | 4 | 4 | 4 | 4 |
| **Volume of distilled water (ml)** | 5 | 4.49 | 4.48 | 4.47 | 4.46 | 4.45 |
| | Incubate at room temperature for 30 minutes | | | | | |
| **Absorbance at 738 nm** | 0 | 0.125 | 0.310 | 0.560 | 0.757 | 0.852 |

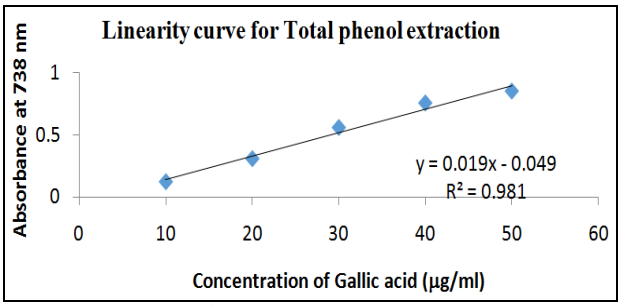



**Fig. 1 Linearity curve for Total phenol Extraction**

### One Variable at Time (OVAT) Analysis of Total Phenol Extraction:

### Effect of Quantity of wood on Total phenol extraction:

Based on the analysis of quantity of wood on total phenol extraction, five Percentage of quantity of wood was evaluated. The highest total phenol extraction was found in shows the best result of 0.044 µg/ml of phenolic content. For the central value optimization 5% is taken as the central value.

**Table 6. Effect of Quantity of wood on Total phenol extraction**

| S. No | Quantity of wood | Total phenolic content (µg/ml) |
|---|---|---|
| $T_1$ | 1 % | 0.020 |
| $T_2$ | 2 % | 0.025 |
| $T_3$ | 3 % | 0.033 |
| $T_4$ | 4 % | 0.039 |
| $T_5$ | 5 % | 0.044 |

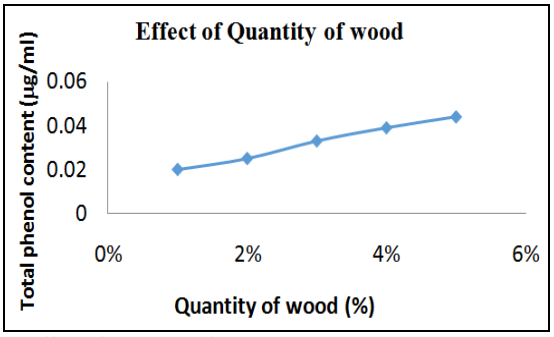



**Fig. 2 Effect of Quantity of wood on Total phenol extraction**

### Effect of temperature on Total phenol extraction

Based on the analysis of Temperature on total phenol extraction, five different temperatureof extraction are optimized. The highest Temperature of wood shows the best result of 0.712µg/ml of phenolic content. For the central value optimization 60°C is taken as the central value.

**Table 7. Effect of temperature on Total phenol extraction**

| S. No | Temperature | Total phenolic content (µg/ml) |
|---|---|---|
| $T_1$ | 20°C | 0.236 |
| $T_2$ | 30°C | 0.548 |
| $T_3$ | 40°C | 0.694 |
| $T_4$ | 50°C | 0.701 |
| $T_5$ | 60°C | 0.712 |
| $T_6$ | 70°C | 0.681 |

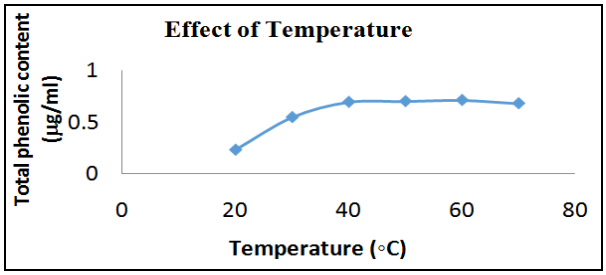



**Fig. 3 Effect of temperature on Total phenol extraction**

## Effect of Extraction time on Total phenol extraction:

Based on the analysis of Time on total phenol extraction, five different extraction time are optimized. The highest incubated time of wood shows the best result of 0.718 µg/ml of phenolic content. For the central value optimization 4 days is taken as the central value.

**Table 8. Effect of Extraction time on Total phenol extraction**

| S. No | Extraction time | Total phenolic content (µg/ml) |
|---|---|---|
| $T_1$ | 1 days | 0.432 |
| $T_2$ | 2 days | 0.508 |
| $T_3$ | 3 days | 0.626 |
| $T_4$ | 4 days | 0.718 |
| $T_5$ | 5 days | 0.682 |

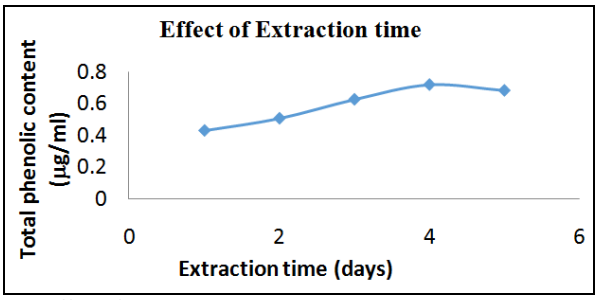



**Fig.4 Effect of incubation time on Total phenol extraction**

## Response for Constructed Model:

The total phenol extraction response of CCRD test and corresponding predicted values were given in Table 8.

**Table 9. Experimental and predicted values of Total phenol extraction from CCRD test**

| Run | Actual Values | | | Total phenol Extraction | |
|---|---|---|---|---|---|
| | A (%) | B(°C) | C (days) | Experimental | Predicted |
| 1 | 5.00 | 50.00 | 5.00 | 0.056 | 0.05 |
| 2 | 4.00 | 60.00 | 3.00 | 0.061 | 0.06 |
| 3 | 5.00 | 50.00 | 3.00 | 0.059 | 0.06 |
| 4 | 6.00 | 70.00 | 4.00 | 0.052 | 0.05 |
| 5 | 6.00 | 60.00 | 5.00 | 0.047 | 0.05 |
| 6 | 6.00 | 60.00 | 3.00 | 0.046 | 0.05 |
| 7 | 6.00 | 50.00 | 4.00 | 0.05 | 0.05 |
| 8 | 5.00 | 60.00 | 4.00 | 0.066 | 0.07 |
| 9 | 4.00 | 70.00 | 4.00 | 0.062 | 0.06 |
| 10 | 5.00 | 60.00 | 4.00 | 0.069 | 0.07 |
| 11 | 5.00 | 70.00 | 5.00 | 0.057 | 0.06 |
| 12 | 5.00 | 60.00 | 4.00 | 0.065 | 0.07 |
| 13 | 4.00 | 50.00 | 4.00 | 0.063 | 0.06 |
| 14 | 5.00 | 60.00 | 4.00 | 0.067 | 0.07 |
| 15 | 4.00 | 60.00 | 5.00 | 0.059 | 0.06 |
| 16 | 5.00 | 70.00 | 3.00 | 0.058 | 0.06 |
| 17 | 5.00 | 60.00 | 4.00 | 0.066 | 0.07 |

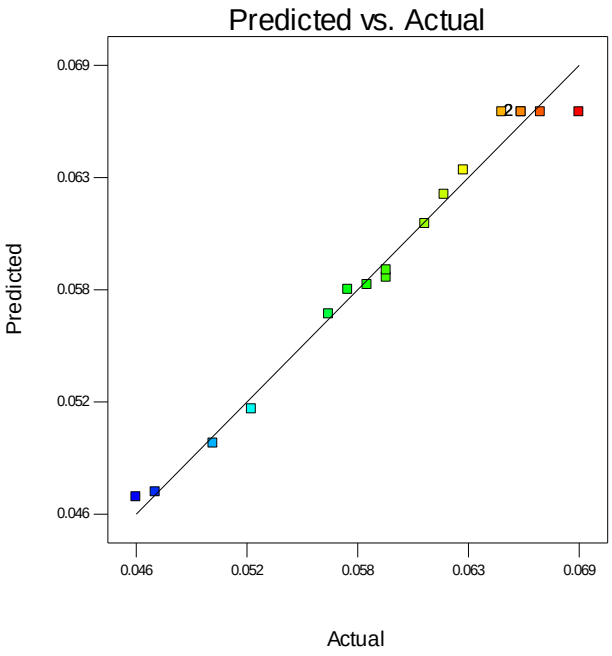



**Fig. 5 Diagnostic plots for extraction process of predicted value *vs.* observed value for total phenol extraction**

Each of the variables was assessed as experimental results as shown in the table9 were fitted to a second-order polynomial equation by applying multiple regression analysis for total phenol extraction (using statistical software package Design- Expert 10.0). The model equation representing the total phenol extraction as quantity of wood (A), Temperature (B), Time(c). They are presented in terms of actual factors as follows:

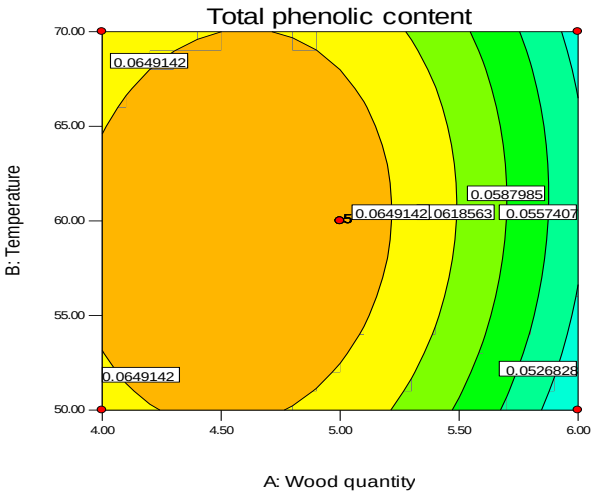
$Y=-713.60326+12.58469A+17.69156B+25.92016+1.14153D-0.44812AB-0.05031AC-9.45875\times1010^{-3}$ AD-$0.12844BC+0.020594BD+8.59375\times10^{-4}$ CD-$0.068510A^2$-$1.1638B^2$-$0.2959C^2$

**Table 10. Analysis of variance of response surface method to predict the phenol extraction**

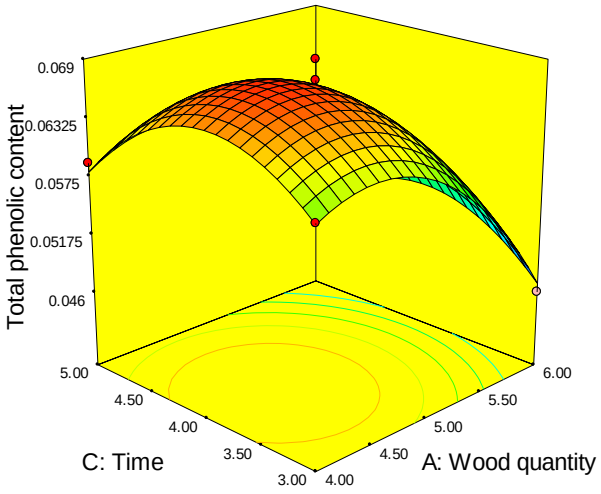
| Source | Sum of squares | Df | Mean squares | F-value | p-value prob>F | |
|---|---|---|---|---|---|---|
| Model | 7.716E-004 | 9 | 8.573E-005 | 48.20 | < 0.0001 | **Significant** |
| A-Wood quantity | *3.125E-004* | *1* | *3.125E-004* | 175.70 | < 0.0001 | |
| B -Temperature | 1.250E-007 | 1 | 1.250E-007 | 0.070 | 0.7986 | |
| C-Time | 3.125E-006 | 1 | 3.125E-006 | 1.76 | 0.2266 | |
| AB | 2.250E-006 | 1 | 2.250E-006 | 1.27 | 0.2978 | |
| AC | 2.250E-006 | 1 | 2.250E-006 | 1.27 | 0.2978 | |
| BC | 1.000E-006 | 1 | 1.000E-006 | 0.56 | 0.4778 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| $A^2$ | 2.093E-004 | 1 | 2.093E-004 | 117.66 | < 0.0001 | |
| $B^2$ | 3.301E-005 | 1 | 3.301E-005 | 18.56 | 0.0035 | |
| $C^2$ | 1.671E-004 | 1 | 1.671E-004 | 93.96 | < 0.0001 | |
| Residual | 1.245E-005 | 7 | 1.779E-006 | | | |
| Lack of Fit | 3.250E-006 | 3 | 1.083E-006 | 0.47 | 0.7188 | **not significant** |
| Pure Error | 9.200E-006 | 4 | 2.300E-006 | | | |
| Cor total | 7.840E-004 | 16 | | | | |

Based on the responses of 17 experiments and ANOVA significance, the best optimal condition of total phenol extraction process was determined as quantity of wood 4.55 %, temperature 59.24°C and extraction time 3.92 days with extraction yield 0.958 mg Gallic acid equivalents (GAE)/g of dry powder. Comparing to Optimized condition for phenol extraction from olive leaves which includes solvent concentration of 80% for ethanol and extraction temperature of 40°C with a yield of polyphenols with 51 mg EAG g-1 dp. [10].

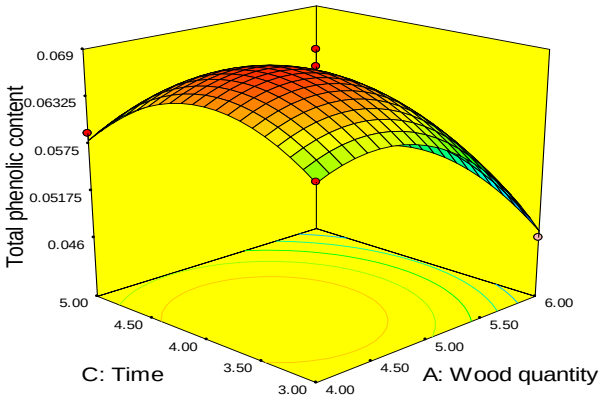



4.6(a)

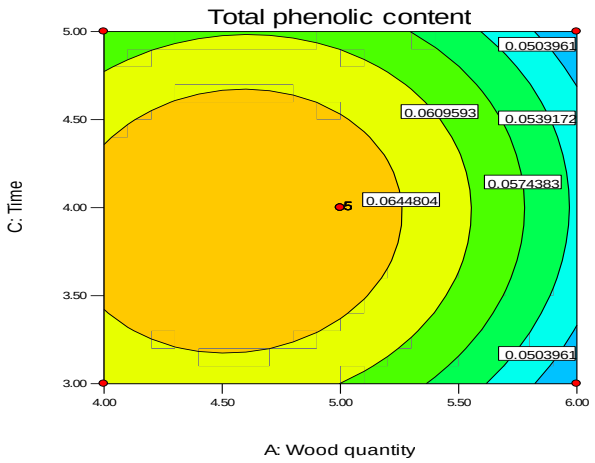



4.6(b)

**Fig.6(a) contour and (b) surface plots for total phenol extraction with respect to Wood quantity and time**

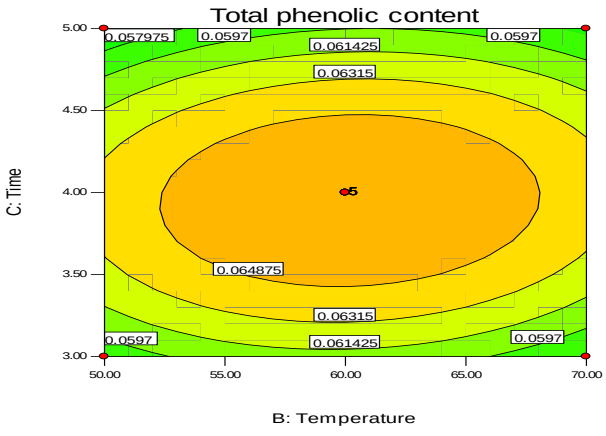



4.7(a)

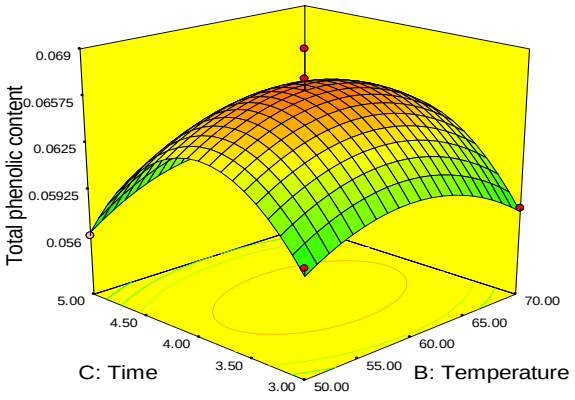



4.7 (b)

**Fig. 7(a) contour and (b) surface plots for total phenol extraction with respect toTime and wood quantity**




4.8(a)




4.8(b)

**Fig.8(a) contour and (b) surface plots for total phenol extraction with Respect to Temperature and time**

## Thin Layer Chromatography:

TLC is one of the techniques for identifying the natural compounds present in the extracts. It is carried out by using silica gel plate were cut with ordinary house hold scissors. The solvent system used in the TLC analysis was chloroform/methanol 4:1.After complete dilution of the solvent; the spots were identified by passing iodine vapor. The $R_F$value of active compound in the crude extracts of benzene, chloroform was determined as 0.667. The determined value was compared the TLC results of 0.85[11]. (Fig.9)

*Rf* = 0.0667

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{distance travelled by the solvem fornt TLC plates}}$$

**Fig. 9 Results of TLC plates**

## GC-MS Analysis:

GC-MS analysis was used to determine the volatile components present in the wood extract. The gas chromatogram of the extract is depicted in the figure 11. The mass spectral patterns of the GC eluents indicate the presence of 2 major compounds. Based on theLibrary search results the compounds were identified as Tri chloro methane and di methyl sulfoxide. (Fig.10)

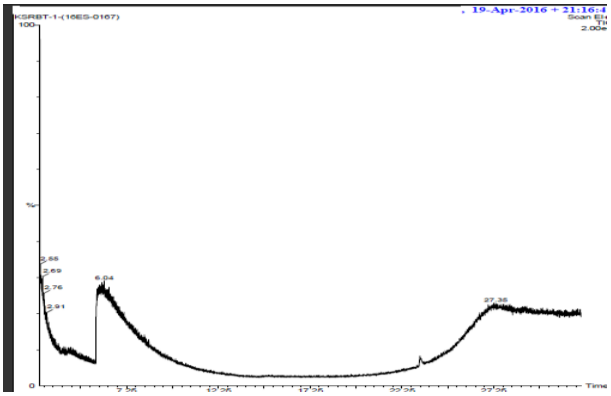


**Fig. 10 Gas chromatogram of wood extract**

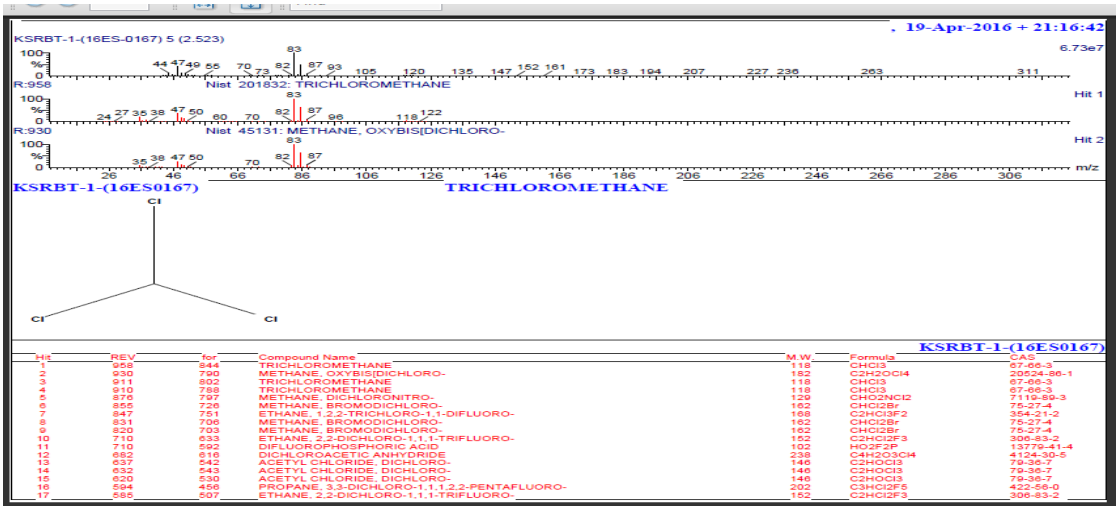
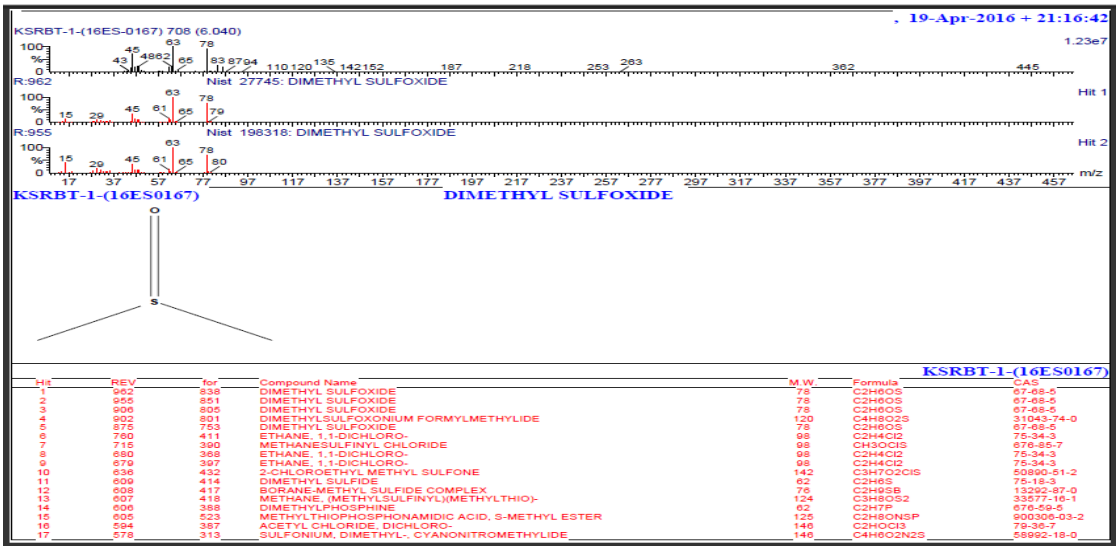



**Fig. 11 (a) Mass spectrometry results aligned with library search**




| Hit | REV | for | Compound Name | M.W. | Formula | CAS |
|---|---|---|---|---|---|---|
| 1 | 962 | 838 | DIMETHYL SULFOXIDE | 78 | C2H6OS | 67-68-5 |
| 2 | 955 | 851 | DIMETHYL SULFOXIDE | 78 | C2H6OS | 67-68-5 |
| 3 | 908 | 805 | DIMETHYL SULFOXIDE | 78 | C2H6OS | 67-68-5 |
| 4 | 902 | 801 | DIMETHYLSULFOXONIUM FORMYLMETHYLIDE | 120 | C4H8O2S | 31043-74-0 |
| 5 | 875 | 753 | DIMETHYL SULFOXIDE | 78 | C2H6OS | 67-68-5 |
| 6 | 760 | 411 | ETHANE, 1,1-DICHLORO- | 98 | C2H4Cl2 | 75-34-3 |
| 7 | 715 | 390 | METHANESULFINYL CHLORIDE | 98 | CH3OClS | 676-85-7 |
| 8 | 680 | 368 | ETHANE, 1,1-DICHLORO- | 98 | C2H4Cl2 | 75-34-3 |
| 9 | 679 | 397 | ETHANE, 1,1-DICHLORO- | 98 | C2H4Cl2 | 75-34-3 |
| 10 | 636 | 432 | 2-CHLOROETHYL METHYL SULFONE | 142 | C3H7O2ClS | 50890-51-2 |
| 11 | 609 | 414 | DIMETHYL SULFIDE | 62 | C2H6S | 75-18-3 |
| 12 | 608 | 417 | BORANE-METHYL SULFIDE COMPLEX | 76 | C2H9SB | 13292-87-0 |
| 13 | 607 | 418 | METHANE, (METHYLSULFINYL)(METHYLTHIO)- | 124 | C3H8OS2 | 33577-16-1 |
| 14 | 606 | 388 | DIMETHYLPHOSPHINE | 62 | C2H7P | 676-59-0 |
| 15 | 605 | 523 | METHYLTHIOPHOSPHONAMIDIC ACID, S-METHYL ESTER | 125 | C2H8ONSP | 900306-03-2 |
| 16 | 594 | 367 | ACETYL CHLORIDE, DICHLORO- | 146 | C2HOCl3 | 79-36-7 |
| 17 | 578 | 313 | SULFONIUM, DIMETHYL-, CYANONITROMETHYLIDE | 146 | C4H6O2N2S | 58992-18-0 |

**Fig.12 (b) Mass spectrometry results aligned with library search**

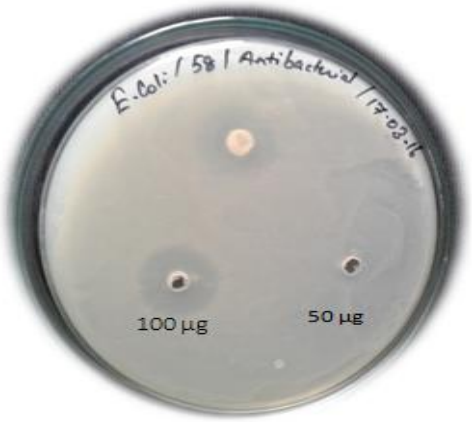
## Anti-Bacterial Activity:

The antibacterial activity of ethanolic extract was assayed against four microorganisms namely *E. coli*, *B.subtilis*, *S.aureus* and *K. pneumoniae* in two different concentration 50 µl and 100 µl. Based on the result the ethanolic extract shows highest antibacterial activity against *E. coli and S. aureus* with 4mm and 5 mm at 100 µl.

**Table 11. Antibacterial effect of wood extract on different micro organisms**

| S. No | Organism | Antibacterial activity in different concentration of phenolic extract | |
|---|---|---|---|
| | | 50 µl | 100 µl |
| 1 | *E. coli* | 1 mm | 4mm |
| 2 | *B. subtilis* | Nil | Nil |
| 3 | *S.aureus* | 1mm | 5mm |
| 4 | *K.pneumonia* | Nil | Nil |


**(b)**




**(a)**


**(c)**


**(d)**

**Fig.13 Anti bacterial activity of ethanolic extract against different microorganisms**

a) *E.coli*, b) *B.subtilis*, c) *S.aureus*, d) *K. pneumoniae*

## CONCLUSION:

The total phenolic content of *Azadirachta indica* wood was extracted using ethanol as solvent. Using RSM and CCRD, optimization of influencing factors such quantity of wood, temperature and extraction time in total phenol extraction were determined as 5% for quantity of wood, 50°C for temperature and 4 days for incubation time as central values. Based on the central values, 17 experiments were designed and experimentally conducted. The responses of 17 experiments, the best optimal condition of total phenol extraction process was determined as quantity of wood 4.55 %, temperature 59.24°C and extraction time 3.92 days with extraction yield 0.958 mg Gallic acid equivalents (GAE)/g of dry powder. The phenolic extract quality was evaluated using thin layer chromatography and GC-MS analysis. The extract shows good anti-bacterial activity against *E.coli* and *S.aureus.* The extraction process has to be further optimized for industrial uses using different solvent.

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