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NUTRITIONAL PROFILE, ANTIOXIDANT AND ANTIPROLIFERATORY POTENTIAL OF INDIGENOUS RED RICE VARIETY *Rakthashali*

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ABSTRACT

***Rakthashali* is an indigenous rice variety which have high nutritional and therapeutic properties. *Rakthashali* rice is rich in proteins and minerals. Calcium content of *Rakthashali* rice was found to be 5.69 mg/100g and the iron content was observed to be 1.67 mg/100g. Rice flour and rice bran showed high antioxidant and cell cytotoxic properties. IC_{50} values for scavenging DPPH, superoxide and hydroxyl were 8.50, 25.4 and 1.72 for rice and 22.5, 49.28 and 2.47 µg/ml for rice bran, respectively. Total phenol and flavonoid contents were also high. Results of cytotoxic assay (MTT) in MCF-7 showed that the cell viability was reduced to a maximum of 23.76 per cent for rice bran extract. *Rakthashali* rice variety showed high antioxidant potential and possesses antiproliferatory property against adenocarcinoma cell line (MCF-7). *Rakthashali* rice variety could be exploited as one of the potential sources of antioxidants and in preventing various life style diseases.**

Keywords: Antioxidant activity, Antiproliferative activity, Flavonoid, phenol, *Rakthashali* rice bran, Rice flour

Rakthashali is an indigenous pigmented rice variety, which is considered as a medicinal rice variety among tribes. *Rakthashali* is a unique grain plant in the *Oryza* genus indigenous to Kerala, has high nutritional potential and is widely used in ayurvedic treatments. Now-a-days, pigmented rice varieties have received great attention owing to their high content of polyphenols, minerals, vitamins and numerous biological activities. *Rakthashali* is reported to be good for health, skin, eyesight, diuretic and it has pharmacological and clinical traits with anti-bacterial, anti-diarrheal, anti-dysenteric, anti-fungal, anti-inflammatory, antioxidant, anti-thyroid, anti-viral, anti-tumor and anti hypercholesterolemic activities. It also stimulates radical scavenging effects. It is traditionally considered as good for fevers and ulcers. Medicinal rice varieties are relatively high in carbohydrate, protein, minerals and vitamins. The bran fraction of rice is distinct from other cereal grains which contains a significant amount of natural phytochemicals including sterols, gamma oryzanol, phenolics, flavonoids, tocopherols, ferulic acid, phytic acid and tocotrienols which have free radical scavenging activity. In general, rice bran contains numerous nutrients, minerals and vitamins, as well as health-promoting bioactive compounds. Due to the antioxidant action of the various phytochemicals present in rice grain and rice bran, it is drawing immense interest in research world as inhibitors of cancer cell proliferation. In the recent years, consumer preferences have shifted towards pigmented types which are having high nutritional and therapeutic properties. The present study investigates the nutritional and therapeutic properties of medicinal rice variety *Rakthashali*.

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MATERIALS AND METHODS

Indigenous medicinal rice variety *Rakthashali* was selected for the study and was procured from farmer's field. The paddy grains were dehulled in rubber roll sheller. The whole dehulled grains of the selected variety were powdered and sieved to get fine rice flour. For collecting rice bran, another portion of the whole dehusked grains were subjected to polishing (9 % DOM) by using a lab level mini rice miller for 30 seconds. The polishing was collected and sieved to get fine rice bran. All the quality evaluation studies were conducted in the whole grain rice flour.

Nutritional composition

The moisture, starch, total carbohydrates, protein, total fat and minerals like calcium, iron, zinc and phosphorous were evaluated in the dehusked rice and also in rice bran. Moisture content of rice and rice bran was estimated by the method of A.O.A.C[1]. The moisture content was calculated from the loss in weight during drying and expressed in percentage. The total starch and carbohydrate contents of the samples were estimated colorimetrically using anthrone reagent (A.O.A.C, 2012; Sadasivam and Manickam, 1992). A graph was prepared using serial dilutions of standard glucose. From the standard graph, the amount of total carbohydrates present in the sample was estimated and expressed in grams. Protein and Fat contents were

estimated by adopting standard method of (A.O.A .C, 2012). The nitrogen content obtained was multiplied with a factor of 6.25 to get the protein content and expressed in percentage. Calcium, zinc and iron content were estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982). The phosphorus content was analysed colorimetrically (Jackson, 1973) which gave yellow colour with nitric acid vandate molybdate reagent and was expressed in mg/100 g.

In vitro digestibility of starch

Starch digestibility was estimated based on standard procedure (Satterlee *et al.,* 1979) One gram of the sample was powdered and gelatinised in 100 ml water and boiled for one hour and filtered. One ml of gelatinised solution was taken and 1 ml of the enzyme solution (saliva diluted with equal quantity of water) was added. The mixture was incubated at 37°C for 1-2 hours. The reaction was stopped by adding 1 ml of NaOH. Later, glucose was estimated and *in vitro* digestibility of starch was computed.

In vitro availability of minerals

Rice and rice bran was extracted with 0.03N hydrochloric acid by shaking the contents at 37˚C for 3 hours. The clear extract obtained after filtration with Whatman No.42 filter paper was oven dried at 100˚C and wet acid digested. The amount of the HCl extractable, calcium, zinc, iron and phosphorus in the digested sample were determined by the methods as described for the estimation of minerals.

$$\text{Mineral extractability} = \frac{\text{Mineral extractability in 0.03N HCl}}{\text{Total mineral}} \times 100$$

Assessment of antioxidant and antiproliferatory activities of rice and rice bran

Antioxidant properties like free radical scavenging activities and antiproliferatory studies in rice flour and rice bran were analyzed.

Antioxidant properties

The rice and rice bran was dried and powdered using grinder and sieved. About 10 g of the powder was weighed and extracted with 100 ml methanol by mechanical stirrer at 48 hours. The extract was filtered using whatman No. 1 filter paper and evaporated to dryness. The residue obtained was used for analysing total flavonoid content, total phenolic content, antioxidant activity, scavenging activities against various free radicals, and also for antiproliferatory activity against cancer cell lines.

Total flavonoid content

Total flavonoid content was estimated in the methanolic extract of *Rakthashali* rice flour and rice bran (Chang, 2002) andexpressed as µg/mg Quercetin equivalent (QE) of dry extracts.

Total phenolic content

The total phenolic content in the methanolic extracts of *Rakthashali* was determined by using Folin-Ciocalteu reagent following a slightly modified method (Ainsworth, 2007). The content of the phenolic compounds was expressed as µg/mg gallic acid equivalent (GAE) of dry extracts.

DPPH radical scavenging assay

The methanolic extract of the rice flour and rice bran was tested for its scavenging activity against the stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) by the method (Aquino, 2001). The reaction mixture was incubated in the dark condition for 20 minutes and the absorbance was measured at 517nm. The percentage inhibition was calculated and concentration needed for IC_{50} was estimated.

Superoxide radical scavenging assay

Superoxide scavenging activity of the extract was determined by nitro blue tetrazolium (NBT) reduction method (Mc Cord and Fridovich, 1969). The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 560 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and the experimental tubes.

Hydroxyl radical scavenging activity

The hydroxyl radical generated in the Fe3+-ascorbate – EDTA-H_2O_2 system (Fenton reaction) degrade 2-deoxyribose the product of which condense with Thiobarbituric acid Reacting Substances (TBAR's) forming pink coloured complex (Elizabeth and Rao, 1990). The absorbance was measured at 532 nm against an appropriate blank solution. Vitamin C was used as a positive control in this assay.

Ferric reducing antioxidant power assay (FRAP)

The antioxidant capacity of rice flour and rice bran were estimated according to a standard procedure (Pulido *et al.,* 2000) with some modifications. The method measures the ferric reducing ability (FRAP). The effective concentration was evaluated by comparing the absorbance values of control and experimental tubes.

Total antioxidant activity

The total antioxidant capacity (TAC) of rice flour and rice bran was determined by phosphomolybdate assay using standard procedure (Preito, 1999). Ascorbic acid was used as a standard. The mixture was allowed to reach room temperature when its absorbance was recorded at 765 nm. Blank was run using the same procedure but containing an equal volume of methanol in place of the samples. The antioxidant capacity was reported as µg of ascorbic acid equivalents (AAE) per ml.

Antiproliferatory activities

Antiproliferative assay in cell lines using MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] assay was done according to the standard procedure (Sun *et al*., 2005) separately in rice and rice bran.

Cell line and maintenance

Human Adenocarcinoma cell line (MCF-7) was purchased from National Centre for Cell Sciences, Pune. The cells were maintained in Dulbecco's Modified Eagle Media (DMEM), with 10% FBS and 100 U/L penicillin and streptomycin under standard concentration.

Cytotoxicity analysis

Approximately, $1x10^6$ cells/mL of MCF-7 were seeded in flat bottomed 48 well plates containing 0.25 mL DMEM. The cells at 80 – 90 % confluency were incubated with different concentrations of rice flour and rice bran extracts (25 – 125 µg/ml) for 48 h. After treatment, 20 µl of 5 mg/ml MTT (3-(4, 5 – dimethyl-2-thiazoyl)-2, 5 – diphenyl – 2 H - tetrazolium bromide) was added to all wells and further incubated for 4 h. The formazan crystals formed were dissolved with 500 µl of dimethyl sulphoxide as solubilizing reagent. Absorbance of the coloured product was measured at 570 nm. Cell viability was then calculated by comparing with the absorbance of control well.

Statistical analysis

All experiments were performed three times. All data were presented as mean ± standard deviation of the mean. The data were recorded and analysed as completely randomised design. The quality evaluation of rice, rice flour and rice bran and the antioxidant and antiproliferative properties of rice flour and rice bran were compared based on standard deviation. Differences between means were considered statistically significant at 5% level ($p<0.05$).

RESULTS AND DISCUSSION

The nutritional composition of rice grain is a major parameter influencing the quality of rice grains. The chemical and nutritional qualities of *Rakthashali* rice and rice bran like moisture, starch, carbohydrate, protein, total fat, energy, calcium, zinc, iron and phosphorus were evaluated. The results on the chemical and nutritional qualities of rice and rice bran are presented in Table-1.

Moisture content is an important parameter and the moisture content in *Rakthashali* rice was found as 9.3% and in rice bran it was 12.3%. Moisture content of 13 per cent and 11.6 per cent were observed in *Njavara* yellow and *Najavra* black respectively (Reshmi, 2011). In another study, Deepa (2018) found that the moisture content of 13.1% in *Njavara* rice which was higher when compared to moisture content of rice in this study. Moisture content may vary due to the influence of environmental conditions at the time of harvest. Moisture content is a critical factor which determine the quality parameters of the grain especially the shelf life qualities.

Generally, starch forms 90% of rice by weight (Lisle *et al*., 2000). In the present study, the starch content of *Rakthashali* rice and rice bran was found to be 71.3% and 23%, respectively. According to Reshmi (2012) the starch content of *Njavara* yellow and black varieties ranged between 74.5–76.1 g/100g and is observed to be slightly higher than the starch obtained in this study. In commonly used varieties like *Jyothi* and *Uma*, the starch content was observed to be 63.1% and 61.2%, respectively (Chandhni, 2015).

The carbohydrate content of *Rakthashali* rice and rice bran was found to be 65.00% and 46.08%, respectively whereas in *Njavara* rice, 73.5% of carbohydrate content was observed (Reshmi, 2012). The carbohydrate content obtained for rice varieties *Jyothi* and *Uma* were 75.60% and 71.45%, respectively (Chandhni, 2015).

Protein content of *Rakthashali* rice and rice bran was found to be 11.60% and 11.36%, respectively. The protein content of 11.80% was obtained for *Njavara* yellow, and for *Njavara* black variety, it was of 12.15% which is similar to the present study (Reshmi, 2012). In traditional rice varieties *Jyothi* and *Uma* the protein content of 7.5 and 7.7 g/100g was noticed.

In the present study, the fat content was found to be 3.68 g/100g for *Rakthashali* rice and 5.70 g/100g for rice bran respectively. Deepa *et al*. (2018) reported that the fat content of *Njavara* was 2.48 g/100g which is slight lower compared to the fat content of the present study. *Jyothi* and *Uma* obtained a fat content of 0.42 and 0.35 g/100g respectively. A lower fat content of 1.30 g/100g and 1.92 g/100g was found in *Jyothi* in studies conducted by Lakshmi (2011) and Sathyan (2012), respectively.

Table 1. Nutritional qualities of *Rakthashali* rice variety

Nutritional qualities	Rice	Rice bran
Moisture (%)	9.30 ±0.44	12.25 ±1.98
Protein (g/100g)	11.60 ±1.17	11.36 ±0.88
Fat (g/100g)	3.68 ±1.00	5.70 ±0.955
Fibre (g/100g)	9.08 ±0.01	18.59 ±0.30
Starch (g/100g)	65.00 ±1.01	23.04 ±0.46
Carbohydrate (g/100g)	71.25 ±0.85	46.08 ±1.33
Energy (Kcal)	339.52 ±1.52	281.06 ±1.96
Minerals (mg/100g)		
Calcium	5.69 ±1.5	12.1 ±1.89
Zinc	1.42 ±1.16	0.88 ±0.16
Iron	1.67 ±0.83	31.01 ±1.98
Phosphorus	352.00 ±3.00	116.00 ±2.00
In vitro digestibility of starch (%)	60.32 ±0.04	18.07 ±0.19
In vitro availability of minerals (%)		
Calcium	70.81 ±1.16	65.41 ±1.40
Zinc	46.65 ±1.09	41.76 ±1.09
Iron	67.04 ±0.93	61.11 ±1.28
Phosphorus	56.32 ±0.91	40.07 ±1.03

Values are Mean±SD

Calcium content of *Rakthashali* rice was found to be 5.7 mg/100g and for rice bran it was 1.2mg/100g. But a higher calcium content of 12.2 and 12.8mg/100g was reported for yellow *Njavara* (Reshmi (2011). Deepa *et al*. (2018) also observed a higher calcium content of 11.6 mg/100g in *Njavara*. Sathyan (2012) observed a calcium content of 5.9 mg/100g which was at par with the calcium content of *Rakthashali* observed in this study.

In the present study, the content of zinc in rice and rice bran was 1.4 mg/100g and 0.9 mg/100g, respectively. The zinc content of 3 mg/100g was found in *Irri-6* rice variety (Ahuja *et al.,* 2008). The zinc content of 0.9 mg/100g was reported in *Njavara* black variety and 0.9mg/100g in *Njavara* yellow variety. It was lower compared to the zinc content obtained in the present study. In common table rice varieties, the zinc content was 1.1 mg/100g (*Jyothi*) and 1.1 mg/100g (*Uma*) (Chandhni, 2015)

In the present study, the iron content was observed to be 1.7 mg/100g and 0.3 mg/100g in *Rakthashali* rice and in rice bran, respectively. Data revealed that iron content in *Njavara* rice was 1.9 mg/100g. The iron content of 0.6 mg/100g was noticed in variety *Jyothi* (Nadh, 2018). The iron content in *Njavara* black and yellow as 0.4 mg/100g (Reshmi, 2012), which was observed to be lower than the iron content observed in this study.

The phosphorus content of *Rakthashali* rice was found to be 352 mg/100g and for rice bran it was of 116 mg/100g. In line to this, Deepa *et al*. (2018) revealed that the phosphorus content of *Njavara* was 354 mg/100g. The iron content of 352.40 mg/100g and 351.40 mg/100g in Njavara black and yellow varieties, respectively was also reported. A phosphorus content of 133.2 mg/100g and 101.35 mg/100g was noticed in *Jyothi* and *Uma* varieties, respectively which was lower compared to the phosphorus content of *Rakthashali*.

The *in vitro* digestibility of starch observed in *Rakthasali* rice was 60.32 ± 0.04 per cent. A lower value of 18.07 ± 0.19 per cent was observed for rice bran of same variety. The *in vitro* availability of calcium, zinc, iron and phosphorus was found as 70.81 ± 1.16 per cent, 46.65 ± 1.09 per cent, 67.04 ± 0.93 per cent, and 56.32 ± 0.91 per cent, respectively. Rice bran obtained lower *in vitro* availability of calcium, zinc, iron and phosphorus which were 65.41 ± 1.40 per cent, 41.76 ± 1.09 per cent, 61. 11 ± 1.28 per cent and 40.07 ± 1.03 per cent, respectively.

In the present study, total flavonoid content in the methanolic extract of rice flour and rice bran was estimated and found as 90.5 mg QTE/g for rice flour and 106.9 mg QTE/g for rice bran extract. According to Rao *et al*. (2010) the total flavonoid content of *Vasumati, Yamini, Jyothi* and *Njavara* rice bran was 1.68, 2.57, 5.33 and 8.5 mg QTE/g, respectively.

Table 2. Total phenol and total flavonoid content

Sample	Total phenol (mg GAE/g extract)extract	Total flavonoids (mg QTE/g extract) extract
Rice flour	3.8 ±0.04	90.5 ±1.4
Rice bran	1.9 ±0.14	106.9 ±4.0

The total phenolic content of methanolic extract of rice flour and rice bran was found to be to 3.8 and 1.9 mg QTE/g extract, respectively. The total phenol content of the rice varieties *Njavara* black and *Njavara* yellow were 0.41 and 0.29 mg GAE/g, respectively. A study (Rao *et al*., 2010) reported that the total phenol content in *Vasumati, Yamini, Jyothi* and *Njavara* ranged between 3.27-12.4 mg GAE/g. The phenol and flavonoid content of the present study has been represented in Table 2.

The stable free radical, DPPH was effectively scavenged by rice flour and rice bran extracts. Rice flour and rice bran extracts dose dependently showed DPPH radical scavenging activity within the concentrations of the extracts (5-40 µg/ml). Methanolic extract of rice bran and rice flour showed significant antioxidant activity with IC_{50} values of 22.5 µg/ml and 8.50 µg/ml, respectively (Fig. 1).

Rao *et al*. (2010) reported DPPH scavenging activity in four tested varieties *viz Njavara* (30.85 µg/ml), *Vasumati* (87.72 µg/ml), *Yamini* (70.58 µg/ml) and *Jyothi* (48.88 µg/ml). IC_{50} values of the free fractions of black, red and brown rice bran against DPPH activity were 25, 32 and 51 µg/ml, respectively (Ghasemzadeh, 2018). From the present study, it was reported that DPPH radical scavenging activity of *Rakthashali* rice was higher compared to other rice varieties reported.

Superoxide radicals generated by the photo reduction of riboflavin were effectively scavenged by rice flour and rice bran extract. The scavenging efficacy of rice flour and rice bran was dose dependent with IC_{50} values of 25.4 µg/ml and 49.28µg/ml, respectively. Fig. 2 represents the superoxide radical scavenging activity of rice flour and rice bran.

The IC_{50} value of *Njavara* black and yellow was 48.78 µg/ml and 55.43 µg/ml, respectively which was similar to the findings in the present study. *Harsha* variety had an IC_{50} value of 60 µg/ml which indicates that *Rakthashali* rice variety has greater superoxide radical scavenging activity compared to other rice varieties. The superoxide scavenging activity of the four rice bran samples *viz Njavara, Jyothi, Yamini* and *Vasumathi* in the form of IC_{50} values was 41, 48, 102, 140 µg/ml, respectively (Rao *et al*. (2010).

Hydroxyl radical scavenging efficacy of rice flour and rice bran extracts was estimated in Fe3+ - EDTA – ascorbic acid - H2O2 reaction system wherein hydroxyl radical mediated deoxyribose degradation was assessed based on TBARS formed. The efficacy was found to be in dose dependent manner. The rice flour and rice bran scavenged the hydroxyl radical significantly and the IC_{50} values were found to be 1.72 µg/ml and 2.47 µg/ml, respectively. Fig. 3 represents the hydroxyl radical scavenging activity of rice flour and rice bran. *Njavara* yellow had the IC_{50} value of 46.00 µg/ml and for *Njavara* black it was of 55.68 µg/ml and it was lower when compared to the hydroxyl scavenging activity of *Rakthashali* rice variety.

Antioxidant activity has been proposed to be related to the reducing power. Therefore, the antioxidant potential of methanol extracts of rice flour and rice bran was estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The extracts of rice

***Rakthasali* Paddy** ***Rakthasali* rice -unpolished whole grains** ***Rakthasali* rice bran**

Fig. 1. Rakthasali paddy, whole grains and rice bran

Table 3. IC_{50} values for scavenging activities of the rice flour and rice bran extracts

Antioxidant activity	Rice flour	Rice bran
IC50value (µg/ml)		
DPPH radical scavenging activity	8.50 ±0.196	22.5 ±1.174
Superoxide radical scavenging activity	25.4 ±2.47	49.28 ±2.8
Hydroxyl radical scavenging activity	1.72 ±0.030	2.47 ±0.077
Nitric oxide radical scavenging activity	ND	ND

flour and rice bran have dose dependently shown ferric reducing ability within the range 5- 25 µg/ml and it is represented in Fig. 4. Methanolic rice bran extracts (0.5 mg/ml) of *Vasumathi, Yamini, Jyothi* and *Njavara* showed absorbance values of 0.59, 1.04, 1.93 and 2.98, respectively. The reducing power of the extracts increased with the increasing concentration of the rice extracts. From this Fig. it was shown that *Rakthashali* rice flour and rice bran had high reducing power activity.

The total antioxidant activity shown by rice bran and rice flour extracts was found to be significant at 100µg/ml which is equivalent to the mg/g Vitamin C. Fig. 5 represents the total antioxidant activity of rice flour and rice bran extracts. At the concentration of 100 µg/ml of the methanolic rice bran extracts, the absorbance values of *Vasumathi, Yamini, Jyothi* and *Njavara* were 1.65, 2.21, 2.52 and 2.93, respectively which were lower compared to the total antioxidant activity of *Rakthashali*.

Antiproliferatory activity was separately studied in rice flour and rice bran. In the cytotoxicity analysis towards human adenocarcinoma cell line (MCF-7), up to a concentration of 120 µg/ml, rice flour and rice bran showed the cell viability and was found to be reduced to a maximum of 20.8% and 23.76%, respectively and the results are represented in figure 6. Antiproliferatory activity was higher for rice bran compared to rice flour. In the present study, a noticeable antiproliferatory activity was observed against adenocarcinoma cell line (MCF-7). The antioxidant potential of *Rakthashali* rice and rice bran is conclusively represented in Table 3.

In the cytotoxicity analysis towards human adenocarcinoma cell line (MCF-7), the IC_{50} value detected in rice flour extracts was low, but rice bran showed comparable activity with the loss of cell viability (26.63%). In rice flour extract the loss in cell viability was observed to be 21.18% in the concentration of 25 µg/ml. From this assay, up to a concentration of 120 µg/ml of rice flour and rice bran extracts, no further increase in cytotoxicity towards human adenocarcinoma cell line (MCF-7) was observed. Antiproliferatory activity was comparatively higher for rice bran than for rice flour extract. In this study, a slight antiproliferatory activity was observed against adenocarcinoma cell line (MCF-7) . The normal cell line and the rice flour and rice bran induced cell death was shown in plates 5, 6 and 7, respectively. In this line, Rao *et al*. (2010) reported that *Njavara* rice bran contains noticeable antiproliferative activity against C6 glioma cells.

Rakthashali rice is found to be nutritionally superior with high protein, starch and iron contents. The results suggest that the medicinal rice variety *Rakthashali* can be a promising source of potential antioxidants and anticancer activity. This study revealed that *Rakthashali* rice and rice bran have antiradical activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ferric reducing antioxidant power activity and total antioxidant activity. Indigenous rice varieties are the treasure trove of micronutrients and bioactive components. But their virtue is not yet realized completely and in spite of their rich therapeutic potential they are on the verge of being endangered. This is the first report on the antiradical efficiency of Indian medicinal rice variety, *Rakthashali*.

Authors' contribution

Conceptualization of research work and designing of experiments (AG, ERA), Execution of lab/field experiments and data collection (RMF, PSL); Data analysis and interpretation of results (AG, CLS); Preparation of manuscript (AG, STP, LS).

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